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EXPERIMENTAL ENDOTOXIN SHOCK IN THE DOG

A Thesis

Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of Master of Science (Surgery)

Department of Surgery

by

Nicholas Anthony Rety

Edmonton, Alberta.

September 1964.

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

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A C K N O W L E D G E M E N T S

The help of many towards this project is remembered with gratitude.

To the following my thanks are especially due:

DR. CECIL M. COUVES, for his teaching and constant guidance, while allowing latitude for self-expression.

THE CANADIAN LIFE INSURANCE OFFICERS ASSOCIATION, for a fellowship.

DR. KONSTANTY KOWALEWSKI, for his wisdom, encouragement and co-operation.

DR. CHARLES W. NASH, for his readiness to help and generosity with his time and advice.

MR. ALEX GECZY, whose skill, loyalty and cheerful acceptance of long hours made the experimental work possible.

MRS. SUSAN MOLNAR, for typing this thesis.

My wife BARBARA, for inspiration, patience in the face of a busy schedule and for accepting a large share of the groundwork in the preparation of this thesis.

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Facilis descensus Averno sed revocare gradum
superasque evadere ad auras hoc opus hic labor est.

Virgil.

A B S T R A C T

The clinical entity of endotoxin shock is of pertinent interest to the surgeon, because in spite of newer concepts of therapy, the mortality rate remains high. In fact, the mortality rate has not changed significantly since the condition was first described fifty-five years ago. There is also convincing evidence that it is becoming more prevalent.

Endotoxin shock is a complication of Gram-negative bacteraemia, which most frequently follows surgical procedures but may also arise in non-surgical practice. It is caused by the liberation of a lipoprotein-polysaccharide complex from the bacterial cell wall which directly or indirectly exerts a powerful sympathomimetic influence and may produce the circulatory changes associated with irreversible shock.

The endotoxin-shocked man is not the most suitable subject for the study of this condition. The dog affords a convenient experimental model for the study of the mode of action of endotoxin and for the evaluation of proposed methods of treatment.

In view of the species difference between man and dog, dissimilarities between the clinical picture and the experiment must be minimized to render the results capable of clinical interpretation. It was attempted therefore, to reproduce the manner of onset of endotoxaemia in man by slow infusion of endotoxin. Heretofore, the effects of experimental endotoxaemia were observed only in animals receiving endotoxin by rapid injection.

The almost universal use of anaesthesia in experimental endotoxin shock is another deviation from the clinical parallel, since the patient in endotoxin shock is usually unanaesthetized. Experiments were therefore conducted on unanaesthetized dogs and the results compared with those obtained under anaesthesia.

Adrenergic blockade with phenoxybenzamine protects animals from the lethal effects of endotoxin when used before shock is induced, but it will not prevent irreversibility in established endotoxin shock. Phenoxybenzamine is reported to have a latent period of action before its full blockade is achieved. To eliminate delay in adrenergic blockade phentolamine, an adrenergic blocking agent of rapid onset, but short duration of action was used in addition. Dogs were treated only after the production of endotoxin shock.

In addition to the usual observations on survival rate and survival time, the parameters studied included the arterial blood pressure, pulse pressure, central venous pressure, hind limb venous outflow, arterial pH, $p\text{CO}_2$ and HCO_3 and urine output.

Seventy two experiments were performed.

CHAPTER I

INTRODUCTION

S H O C K - A B R I E F H I S T O R I C A L R E V I E W

Shock associated with sepsis was described by Laennec as long ago as 1831. Boise reintroduced the subject into the literature in 1897 (3). The first to link shock with Gram-negative bacteremia was the German, Jacob, in 1909 (51). Perhaps as a result of the war which soon was to follow, probably because of the lack of adequate interchange of medical information between nations prior to the development of the sophisticated communications of today, and certainly due to the lack of funds for research in the decades following the First World War, the subject of septic shock received scant attention in the medical literature in the next forty years.

The significance of vasoconstriction in shock was recognized in 1879 by Mapother when he observed that the most marked physical change caused by shock is contraction of the arterioles (61).

In 1905 Malcolm (60) stated that contraction of the arteries and arterioles is known to occur as a consequence of every injury, and that shock is associated with an exaggeration of this response. According to him, "a very severe degree of shock may be certainly brought about without any haemorrhage at all" through exaggerated constriction of vessels on the arterial side of the circulation. He urged that the treatment of shock be directed to "preventing the ill effects of local irritation, to relaxing the vessels as soon as possible, and to keeping up the blood pressure in the superficial vessels

until such time as a physiological relaxation takes place." Malcolm's ideas were well conceived, yet he was considered a rebel in his day, since according to teaching current at that time shock was caused by relaxation of the blood vessels in the splanchnic area.

In 1919, Erlanger and his associates (17) found that in experimental shock produced by a variety of methods peripheral resistance invariably increased.

Cannon (7) also recognized the deleterious nature of excessive vasoconstriction in shock at the expense of flow to tissues, when he wrote in 1923: "damming the blood in the arterial portion of the circulatory system when the organism is suffering primarily from a diminished quantity of blood, obviously does not improve the volume flow in the capillaries."

Freeman and his associates (23) in 1938 demonstrated that elimination of sympathetic influence may be beneficial in severe haemorrhage. When normal dogs were bled to a level of 60 mm. Hg systolic pressure, after three and a half hours a spontaneous decline in blood pressure was observed. Reinfusion of the bled volume did not raise the blood pressure or the flow in the hind paw, and the dogs died. With sympathectomized dogs blood pressure could be kept below 60 mm. Hg for six hours. Reinfusion of the bled volume led to complete recovery.

Since the sympathetic nervous system is regarded as one

of the major agencies through which the body maintains homeostasis, it is difficult to envisage its action as deleterious. To this Nickerson (68) replies that "a system which prepares the animal for flight or fight, and which is geared to sustain skeletal muscle function at top efficiency, may not be as useful in the body's attempts to maintain a critical minimum of oxygenation of various tissues, following severe haemorrhage or trauma".

Levy and his group (47) from their studies on traumatic shock, drew the conclusion that sympathetic discharge is protective during the initial assault, but it initiates processes detrimental to survival.

Lillehei and his colleagues (54) ask why the vasoconstrictive response survived through evolution if it is harmful. Their answer is logical enough: the vasoconstrictive response is valuable when no treatment is available and it will, therefore, spare us problems of lesser trauma. It is no more than "Nature's first aid", useful until the doctor comes and administers treatment.

Following World War II, the study of shock was carried aloft on a wave of fresh interest. Of particular interest were the studies of Zweifach and his colleagues (121) who looked to the capillary bed as the logical site for a study of shock involving a progressive deterioration and ultimate failure of the peripheral circulation. They showed that irreversibility to transfusion in haemorrhagic shock is associated with a hypo-reactive state in the capillary bed,

with pooling of trapped blood in some regions of the capillary network which were being bypassed by an active circulation elsewhere in the bed.

Zweifach and his colleagues also showed that the reticulo-endothelial system (RES) was an important determinant of vascular behaviour in shock following trauma, endotoxin and haemorrhage in the experimental animal (119). Blockade of the RES with a large dose of colloid increased the susceptibility of the animal to the effects of trauma, endotoxin and haemorrhage.

Stimulation of the RES with repeated injections of colloid, on the other hand, increased tolerance to shock. Zweifach and his group concluded that there was a basic identity in the shock reactions to trauma, endotoxin and haemorrhage, and that the lethal consequences of these insults are regularly associated with altered behaviour of the RES.

Jarisch once said: "It is a source of regret that the measurement of flow is so much more difficult than the measurement of pressure" (46). Indeed, one of the truly important advances in our understanding and management of shock of various aetiologies was the shift of interest from the blood pressure level to the extent of flow in the tissues. It became increasingly evident that vasoconstriction, while maintaining the blood pressure and the functions of vital organs, also set in motion a sinister train of events, which,

if not checked in time, could lead to death of the animal. With decrease in flow due to vasoconstriction, the pressure relationships across the capillary beds are altered. This promotes loss of fluid from the intravascular compartment, with resultant decrease in plasma volume, which will then accelerate irreversibility. Thus, blood pressure has come to be regarded as a far less useful criterion of shock and a less valuable diagnostic aid (22,23,24,47,54,68,71,73,75,80, 108,113).

Zweifach (116) demonstrated that the micro-circulation showed two discrete phases of behaviour in response to both haemorrhage and trauma. Initially the small blood vessels go through a period of increased vasomotor activity, during which blood flow is restricted to the most central, direct channels. With this type of tissue perfusion aerobic metabolism is still possible, and blood replacement will restore the circulation to normal. If, with the loss of blood, peripheral flow in many regions is reduced below the point where oxidative tissue metabolism can be carried out, or when hypotension persists for several hours, the relative autonomy of the terminal vascular bed becomes the dominant factor, and the initial phase of compensation is superseded by a decompensatory tendency. At this stage the terminal arterioles dilate, the precapillary sphincters open, while the small veins remain contracted. This is the micro-circulatory picture of irreversibility, which was mentioned briefly above in connection with Zweifach's earlier work.

Shorr and his collaborators (91) in 1951 claimed that the vasoactive principles VEM (vasoexcitor material) and VDM (vaso-depressor material) were the chief determinants of vascular behaviour in shock. Working with dogs and rabbits, they showed that VEM is elaborated by the kidney and is responsible for the heightened vasomotor activity during the compensatory phase of shock. Others ascribed this vascular behaviour to the discharge of adrenal corticosteroids, angiotonin, epinephrine and norepinephrine (116). The origin of VDM was shown by Shorr and his group to be in the liver, spleen and skeletal muscle. They believed that the cause of irreversibility was increasing elaboration of VDM from these tissues, with concomitant depression of the mechanism which inactivates VDM. VDM was later identified as ferritin, the metabolism of which is particularly disturbed in the hypoxic liver in shock.

Frank and his colleagues (21) showed that VDM is found in the blood only in association with anaesthesia or morphine. Since unanaesthetized and unsedated dogs were also liable to irreversible shock, these workers doubted that VDM can account for the development of irreversibility to transfusion in experimental haemorrhagic shock.

Another theory of irreversibility to transfusion in shock was elaborated by Fine and his group in 1954 (18,43). The crucial event in shock, they claimed, was hypoxia of the bowel. The devitalized intestinal wall permitted bacteria,

chiefly *E. coli*, to pass from the lumen of the gut to the bloodstream with resultant bacteremia. That bacteremia was the cause of irreversibility was shown by the fact that animals pretreated with antibiotics were protected from a lethal outcome.

Fine and his group demonstrated the presence of the toxic factor in the blood of an irreversibly shocked dog in the following way: when blood from such a donor was transfused into a normal recipient, nothing untoward developed. When, however, the recipient was a dog in reversible shock, the transfusion of shocked blood resulted in irreversibility (88).

Hardy (36) and others (95) reproduced Fine's experiments carefully and found that pretreatment with antibiotics did not significantly affect mortality in haemorrhagic shock.

Lillehei and MacLean (52) also found sterilization of the intestine with antibiotics to be of no value in haemorrhagic and endotoxin shock.

Zweifach and his colleagues (120) also produced findings contradictory to Fine's when they showed that irreversible haemorrhagic shock can be produced in germfree rats in a bacteria-free environment.

The importance of the intestine in the pathogenesis of irreversible shock, was however, further emphasized by Lillehei (48,50) when he showed that the lethal outcome of graded haemorrhage could be prevented by perfusion of the small intestine with arterial blood. Lillehei further

states that haemorrhagic necrosis of the intestine is responsible for a large part of the loss of plasma volume and is a result of ischaemia during shock. In his view the intestinal damage appears to be the cause of irreversible shock in the dog (51).

Although there is a substantial support (119) for the identity of haemorrhagic, traumatic and endotoxin shock, it is likely that shock due to endotoxins and that due to haemorrhage or trauma, are identical only with regard to the final vasoactive elements involved (116) and the behaviour of the microcirculation in the visceral organs (54).

Nevertheless, a brief review of changing concepts of the pathogenesis of shock and irreversibility is of value for the appreciation of circulatory and metabolic events in shock due to endotoxins.

E N D O T O X I N S H O C K.

Endotoxin shock, in spite of growing understanding of its pathogenesis and improvements in its treatment, continues to worry the surgeon, baffle the researcher and kill patients in hospital wards.

Endotoxin shock, writes MacLean (59) is second only to myocardial infarction as the cause of shock in hospitalized patients.

"It is disturbing" - writes Lillehei - "that our increasing knowledge of the mechanisms of endotoxin shock is not reflected in much improvement in the mortality rate of patients suffering from this condition". He states that the present mortality from shock associated with gram-negative bacteremia is over 50%, essentially unchanged in the half century since Jacob described the condition in 1909 (51).

Spink (97) in another series, reports a mortality rate of over 70%, while Altemeier and Cole put it at 61% (3).

Shubin and Weil (92) report 692 cases of bacteremia from 1956 to 1960 at the Los Angeles County Hospital. Of this number 169 developed endotoxin shock, with a mortality rate of 82 per cent.

Endotoxin shock has been defined (97) as a state of peripheral vascular collapse induced by a lipoprotein-polysaccharide complex liberated from the cell wall of gram-negative bacilli, particularly the coliform group. Clein and Couves (10) reported that organisms cultured from infected

foci in endotoxin shock were as follows: *E. coli*, *Staph. aureus*, *Ps. aeruginosa*, *Cl. welchii*, *Aer. aerogenes*, *Pr. vulgaris*, *Haemolytic streptococcus*, *Candida albicans*. *E. coli* represented 35.5% of the total while *Staph aureus* accounted for 32.2%.

Factors predisposing to endotoxin shock, in order of diminishing frequency are:-

- peritonitis
- genito-urinary instrumentation
- infected burns
- biliary surgery
- wound infection
- septic abortion
- extensive surgical procedures in debilitated patients.

Sheriton and his group (90) point out that the diagnosis of gram-negative bacteremia must be considered in obscure post-operative hypotension. The urgency is great: death or complete recovery may depend on the adequacy of treatment in the first 24 hours. They also warn that transient bacteremia must be borne in mind particularly after removal of an indwelling catheter.

Shubin and Weil (92) report the onset of endotoxin shock an average of 16 hours after manipulation in urological practice.

Nor is septic shock confined to surgical practice. It

may complicate malignancies, blood dyscrasias, diabetes mellitus, liver disease, or treatment with antibiotics, corticosteroids or antimetabolite drugs. Ruedy reports 43 cases among non-surgical patients at the Montreal General Hospital during the 18 month period 1st July, 1961 to 31st December, 1962 (92,94).

Clinical endotoxin shock embodies the triad of a septic focus, fever and hypotension. The patient is usually over 45 years of age. The condition commonly presents with chills, followed by a high fever of the order of 103-105 degrees F. The blood pressure may drop abruptly to low levels. The majority of patients with endotoxin shock are pale and cyanotic, with cold, moist skin, clouded sensorium, apathy and listlessness. Some patients, however, have a warm, dry skin, in spite of the profound hypotension. According to MacLean, this suggests that the syndrome of septic shock is not homogeneous and that different degrees of organ perfusion may be present in the two types of patient, or at different times in the same patient (57).

The blood culture is usually positive in endotoxin shock, and there is marked leucocytosis. Metabolic acidosis is usually present. Oliguria or anuria complicates the picture.

In the majority of cases endotoxin shock is refractory to conventional methods of treatment usually applied to shock.

Endotoxins.

According to Rosen (81) endotoxin is associated with the cell wall of gram-negative bacteria of the colonially smooth variety. Endotoxin is a generic term, it does not designate a simple, specific, chemically homogeneous substance. Endotoxins exert similar effects, regardless of the bacterial species from which they are derived. They can be distinguished from each other by their antigenic specificity which is due to the polysaccharide moiety.

Mode of action of endotoxin.

There is a considerable volume of experimental evidence suggesting that gram-negative bacteria cause their lethal effects by means of the ability of their endotoxin to act as a potent sympathomimetic substance or by sensitizing the animal to levels of endogenous sympathomimetic agents which are ordinarily not toxic (28,52).

Thomas (98) quotes Delaunay's experiments with rabbits in which, 15 minutes after intravenous endotoxin administration intense arteriolar constriction developed, interrupted by brief periods of arteriolar dilatation. When shock-producing doses were given to rabbits, the phase of constriction was followed terminally by one of extreme arteriolar dilatation, slowing and stasis of the capillary flow. From these findings Thomas inferred that the systemic effects of endotoxin are associated with a generalized sympathomimetic response.

Thomas quotes other reasons for suspecting that epinephrine, or a substance like it, may be implicated in the reaction to endotoxin:

- both endotoxin and epinephrine produce
 - a) hyperglycaemia, followed by hypoglycaemia
 - b) depletion of liver glycogen
 - c) elevation of blood lactic and pyruvic acid levels
 - d) Leucocytosis
 - e) petechial haemorrhages and focal necroses in the upper G-I tract, mesenteric nodes, spleen and liver.

In his own experiments Thomas demonstrated extensive lesions of haemorrhagic necrosis when epinephrine was injected into rabbit skin within 4 hours of intravenous endotoxin administration. Pre-treatment with phenoxybenzamine, chlorpromazine and cortisone prevented the development of these lesions. Thomas concluded that endotoxin alters the reactivity of blood vessels to epinephrine, so that this hormone becomes a powerful necrotizing agent. He stated that it is possible that this effect represents a basic mechanism in the various intoxicating actions of endotoxin.

Zweifach and his group (122) continued this study with microscopic visualization of the micro-circulation in the rat mesoappendix in vivo and with similar examination of the isolated perfused rabbit ear in vitro. In the rat mesoappendix,

topical application of a threshold dose of epinephrine alone produced transient constriction of the terminal arterioles and precapillaries, with cessation of flow. Caliber and flow returned to normal after 10-20 seconds. When an identical dose of epinephrine was applied following a sublethal dose of endotoxin, intense, widespread vasoconstriction was seen in the small arteries, arterioles, venules and small veins, with complete ischaemia of the capillary bed, lasting several minutes. When the same dose of epinephrine followed a lethal dose of endotoxin, a biphasic vascular response was observed. Reactivity was enhanced initially - though to a lesser degree than after a sublethal dose - for about one hour.

In the second hour the terminal vascular bed, and also the larger arteries and veins, showed a gradual depression of epinephrine reactivity. The animal became ill, the respirations laboured and the mean blood pressure fell to 65-75 mm. Hg. The small arteries and veins, especially the muscular venules, became dilated and filled with blood. The entire capillary bed filled with blood which moved slowly through its ramifications. Petechiae appeared locally.

In the third hour the larger arteries and veins narrowed down again spontaneously and became highly reactive to epinephrine. Narrowing continued until the larger arteries became thread-like. The terminal vessels remained unresponsive and dilated.

As a result, large volumes of blood remained stagnant in the capillaries and venules, with increasing numbers of petechiae dotting their course.

This is the microcirculatory picture which Fine (19) so aptly described as "many dry river beds intermingled with swamps".

In this phase direct circulation was demonstrable only through direct shunts which bypassed the capillary bed.

MacLean (57) quotes evidence that arteriovenous shunts in normal intestine account for only 2-4 per cent of the total flow and that there is no indication that these shunts are increased in shock due to endotoxin.

An interesting observation made by Zweifach was that in this experiment the small veins, which are normally the least reactive of the terminal vessels to epinephrine, became more sensitive than the precapillary arterioles, thus providing a mechanism for impeding capillary circulation and pooling of blood in the collecting venules.

The hypersensitivity of muscular venules and small veins following endotoxin administration is also reported elsewhere (25,40,51,116).

In the study of the microcirculation in the isolated, perfused rabbit ear, other important vascular responses were brought to light. When endotoxin was infused continuously, as opposed to a 20-30 second injection, a sub-threshold dose

of epinephrine produced prolonged intense vasoconstriction.

The response to serotonin was not influenced by endotoxin when tested by the above methods.

Zweifach and his colleagues, therefore, raised the possibility that the haemorrhagic necrosis produced in the skin of the rabbit by Thomas may be a result of the biphasic response pattern to epinephrine and that this represents the basic mechanism of tissue damage in endotoxin shock. It is possible, they say, for some tissues to be sufficiently supplied with epinephrine so as to be placed in jeopardy by endotoxin.

The sympathomimetic action of endotoxin on the bowel and its mesentery in the dog is also reported by Lillehei and MacLean (52,53). They point out that the profound degree of bowel ischaemia, leading to increase in vessel permeability, plasma loss and, eventually, irreversible shock, is a picture not unlike that seen in chronic epinephrine shock. MacLean (57) cites a report by Freeman that infusion of epinephrine in the dog results in irreversible shock characterized by haemorrhagic necrosis in the small intestine. Lillehei and MacLean (52,53) duplicated this experiment and obtained identical results.

Apart from sensitizing blood vessels to epinephrine, it is also held that endotoxin is a sympathomimetic substance itself, when combined with some formed element in the blood, probably the leucocyte (51,54).

The claim that endotoxin administration to an experimental animal causes marked elevations in circulating catecholamine levels has much supporting evidence (2,25,51,54,55). It has also been postulated (2) that the main source of catecholamines after endotoxin is extra-adrenal, presumably from elaboration at sympathetic nerve endings.

There is also evidence that serotonin may be implicated in the harmful effects of endotoxin. Davis and his colleagues (12) reported release of serotonin from platelets in the rabbit following endotoxin injection, and they state that the released serotonin may be responsible for the observed vasoconstriction.

Hinshaw and his group (39) observed increase in the weight and vascular resistance of the isolated perfused lung of the dog when endotoxin was perfused in whole blood or cell-rich plasma. These changes were not seen when the endotoxin containing perfusate was cell-poor plasma, dextran or gelatin. Since serotonin causes entirely similar changes in lung weight and pulmonary vascular resistance, it is possible that the substance in blood or cell-rich plasma necessary for this activity of endotoxin may be serotonin released from platelets.

Thomas and others (100) have found that both serotonin and endotoxin greatly enhance the necrotizing and vasomotor effects of epinephrine and norepinephrine on the small blood vessels. They hypothesize that endotoxin may make serotonin available in and around the blood vessel walls for action

exerted in concert with epinephrine. They found that the combination of epinephrine and serotonin produces the same skin lesion as that produced by epinephrine and endotoxin.

However, specific serotonin antagonists have not been found useful in experimental endotoxin shock (52).

Rubenstein, Fine and Coons (83) carried out studies to localize *E. coli* endotoxin after intravenous injection into dogs, using the method of immunofluorescence. Fluorescent material was widely distributed throughout the walls of the peripheral vascular system. The endothelium of capillaries and veins was frequently involved, while that of the arterioles only rarely. The great vessels were spared. There appeared marked association of endotoxin with leucocytes. It seemed also that the leucocyte acts as a transport mechanism for the endotoxin in diapedesis. Rubenstein and his colleagues inferred from these findings that endotoxin may have a direct action on blood vessel walls.

The work of Schayer (57,85,86) focussed interest on the role of histamine in shock, particularly shock due to endotoxin. He postulated that a homeostatic relationship exists in the body between catecholamines and newly formed histamine. Adrenergic stimulation results in increased histidine decarboxylase activity which augments histamine synthesis. According to Schayer the balance is doomed, since epinephrine is released rapidly from a finite store in the adrenals while histamine is being synthesized at an increasing rate. As a

result the responsiveness to epinephrine is superseded by histamine predominance and vascular damage.

While some of the drugs used successfully in pretreatment of animals against endotoxin shock, are known to have anti-histaminic action (e.g. chlorpromazine, phenoxybenzamine), the value of specific antihistamine drugs has not been established in the condition (52,57).

Hardaway proposed the theory that the crucial mechanism in the causation of both hypovolemic and normovolemic shock is the formation of intravascular thrombi. When he administered *E. coli* endotoxin to dogs he noted immediate disappearance of platelets, gradual disappearance of fibrinogen, factor V and factor VII. He reasoned that the fall in the level of clotting factors was probably due to their use in intravascular coagulation. He further stated that the endogenous activation of heparin and fibrinolysin represents a protective mechanism. Pretreatment of the animal with heparin prevents the formation of intravascular thrombi and promotes survival. Irreversibility occurs when the thrombi remain in place long enough for ischaemic necrosis of the tissues to occur. Hardaway claims that endotoxin shock is due, at least in part, to intravascular coagulation (33,35).

Cavanagh and De Cenzo (8) observed the parallel between endotoxin shock and the generalized Shwartzman phenomenon. In the latter, after "sensitization" by endotoxin, fibrin thrombi occur in the lung, liver and spleen. These are

rapidly removed by the reticuloendothelial system, which suffers temporary blockade as a result. If, within 48 hours another dose, the so-called "provoking dose", of endotoxin is given intravenously, a new wave of fibrinogen-fibrin conversion appears but this time the fibrin thrombi also form in the kidneys, causing ischaemic necrosis of the renal cortices. In the pregnant woman no "sensitizing" dose is required for the generalized Schwartzman reaction to occur, since pregnancy sensitizes both the human and animal subjects to endotoxin (62).

Cavanagh and De Cenzo, therefore, used fibrinolysin in the treatment of endotoxin shock in dogs. They found that fibrinolysin given within 15 minutes after endotoxin significantly increased survival, but it had no protective effect when administered in a later phase of shock.

The clearance of endotoxin from the circulation was investigated by Herring and his colleagues (37) in normal rabbits and rabbits that had been rendered tolerant to endotoxin by the injection of repeated small doses. The endotoxin was labelled with chromium 51. In both groups small doses of endotoxin were cleared within 10 minutes. The group tolerant to endotoxin showed a somewhat higher rate of clearance. There was rapid uptake in the liver, suggesting a more or less direct transfer from the blood to the reticuloendothelial cells. The increased rate of clearance in the tolerant animals may have been due to increased phagocytosis

stimulated by previous exposure to endotoxin. The study also showed that most, if not all, endotoxin in formed elements in the blood is associated with platelets.

There was a marked absence of radio-activity from leucocytes in this experiment. However, the authors state that this may be explained by prompt margination of leucocytes which then would not appear in the blood samples (37).

Gilbert (25) quotes other studies on the clearance of endotoxin. In rabbits after an intravenous injection the plasma level fell to $3/5$ of the immediate post-injection level in one hour, and to $1/12$ after two hours. In mice and guinea pigs 60-70 per cent disappears in 8 minutes, but the remainder persists in the blood for hours. The influence of previous depression or stimulation of the reticuloendothelial system on endotoxin clearance again strongly suggested that this system is active in removal of endotoxin from the plasma.

Endotoxin shock in the dog.

Endotoxin shock in dogs is usually produced by intravenous injection of crude endotoxin prepared from *E. coli* cells, the lethal dose having been arrived at by assay on control dogs (53), or by purified endotoxin prepared and standardized by a manufacturer. The endotoxins prepared by Difco Laboratories, Detroit, Mich., are frequently used in research on this continent.

In all reports read by the writer endotoxin was administered by rapid injection. The dogs are usually anaesthetized with pentobarbital or sedated with morphine.

When a lethal dose of endotoxin is injected intravenously into an adult mongrel dog, within 30-60 seconds there is a precipitous fall in the systemic blood pressure. At the same time the portal venous pressure rises. Within 15-30 minutes the systemic blood pressure rises again approximating the pre-endotoxin level. Thereafter it begins a slow, steady decline. The pulse pressure diminishes concomitantly. Tremor, excessive salivation, vomiting, bloody diarrhoea and terminal convulsions may complicate the picture. There is progressive metabolic acidosis, but the pH of the arterial blood tends to remain at near normal levels until the buffering capacity of the blood is exhausted. Oliguria or anuria is often seen. The plasma volume decreases progressively. Haemoconcentration and haemolysis develop (51,97).

At autopsy the most marked finding is haemorrhagic necrosis of the small intestinal mucosa. Oedema of the small intestinal wall is frequently present. Areas of subendocardial haemorrhage may be found in the heart. According to Lillehei and his associates (51) this picture of endotoxin shock in the dog is identical with that produced by prolonged (4-5 hours) haemorrhagic shock followed by retransfusion, by intravenous injection of epinephrine, 2 mg./Kg., or by occlusion of the superior mesenteric artery for 4 hours.

The hepatic venous sphincter controversy.

The mechanism of the initial precipitous blood pressure drop after endotoxin injection has been the battlefield of conflicting ideas.

In 1906, Mall demonstrated spiral valves in the hepatic veins of dogs. In 1932, Bauer and others described thickening of the muscular coats of hepatic veins toward their orifices into the inferior vena cava. The fibres are arranged longitudinally, but on contraction cause projection of the intima into the lumen, giving a valve-like effect (89).

It has been claimed (57,117) that endotoxin produces spasm of these sphincteric muscle bundles, which then results in obstruction to hepatic venous outflow, impairment of venous return and sudden fall in cardiac output.

MacLean (57) quotes evidence which makes it unlikely that constriction in the hepatic veins is responsible for the hepatic congestion observed. Catheters placed 2 - 3 centimetres into the hepatic veins from the inferior vena cava recorded no elevation of pressure, while simultaneous measurement in the portal vein revealed a rise of pressure from 10 to 40 mm. Hg. Weil and MacLean, as cited by MacLean (57) noted elevation of portal vein pressure in the rat and the rabbit after endotoxin injection. Neither of these animals possesses hepatic vein sphincters.

Thomas and Essex (101) studied the behaviour of the hepatic venous circulation following injections of extracts of

Ascaris suum in the dog. Engorgement of the liver was seen. The size of the organ increased by 50-150 per cent and the portal pressure rose from 7 to 31 centimetres of water. Cannulae passed 9 centimetres into the hepatic veins reflected pressures typical of systemic venous pressure, not those of the portal system. The conclusion was drawn that the mechanism is not a sphincteric action in the hepatic veins, but rather diffuse spasm of the entire hepatic venous side of the liver vasculature. This is even more pronounced in the smaller than in the larger vessels.

MacLean (25,52,57) showed pooling of blood in the liver following endotoxin injection by weighing the organ in vivo. As the weight of the liver increased, the central venous pressure fell. The increase in weight, representing the amount of blood by which the venous return had diminished, could easily account for the degree of shock observed. Liver weight returned to normal in 5-25 minutes. This accounts for the temporary restoration of the blood pressure.

This study also showed increase in intestinal weight in endotoxin shock, especially in the later phase, at which time portal pressure is normal.

The work of Daniel and Prichard (11) has contributed to our knowledge of the functional aspects of blood flow within the liver. They carried out rapid serial angiography to show the passage through the liver of "Thorotrast" injected through a tributary of the portal vein. They showed that on

occasion the superficial regions of the liver may fail to be perfused, while circulation is maintained through deeper parts. When intrahepatic distribution of portal blood flow is restricted in this manner, the portal blood reaches the inferior vena cava more rapidly than when the liver is perfused throughout. Daniel and Prichard did not demonstrate the stimulus inducing restriction of the intrahepatic circulation but suggested that it was a neurovascular mechanism. This study showed in addition that there was no communication between the portal and hepatic venous systems except by way of the sinusoids.

Moore (64) accepts the possibility of portal venous congestion due to a sphincteric mechanism in the hepatic veins, but states in addition that vascular changes at sinusoidal level may be the cause.

Lillehei and his colleagues (51) are emphatic that no sphincteric mechanism exists in the hepatic veins. In support of this contention they state that portacaval shunt does not protect the animal from the intestinal changes due to endotoxin. In their view these sphincters are often used in argument aimed at discounting the dog as a suitable experimental model in relation to man.

Peripheral resistance in endotoxin shock.

MacLean quotes the unpublished data of Hinshaw and his co-workers who found that during the early phase of endotoxin

shock in the dog the total peripheral resistance remained relatively normal. Later there was a decline in total peripheral resistance which contributed to the shock state (57).

Rayner and MacLean (79) found no rise in intestinal vascular resistance in endotoxin-shocked dogs. There was a selective decrease in blood flow to the mucosa of the intestine.

Lillehei and his group (51) hold contrary opinions. They claim that in endotoxin shock peripheral resistance is high. They state in evidence that even isolation and perfusion of the dog's intestine via the superior mesenteric artery - which protects the animal from irreversible haemorrhagic shock - is not protective in endotoxin shock, since in the latter resistance in the superior mesenteric system is even higher and therefore unphysiological pumping pressures are required. They state that blood flow to the bowel in shock is about the same as collateral flow to the bowel after experimental occlusion of the superior mesenteric artery of the dog.

The work of Weil and his group further supports the contention that in shock due to infection systemic resistance is increased (102).

Acidosis in endotoxin shock.

The occurrence of metabolic acidosis in endotoxin shock is well documented (4,10,25,34,57,59,97,108,110). It seems

generally accepted that the development of acidosis in shock is the consequence of inadequate tissue perfusion. Excessive reduction in tissue blood flow due to vasoconstriction results in tissue hypoxia. This in turn encourages the production of lactic acid. There is good correlation between the rise in blood lactate and the decrease in serum bi-carbonate and pH early in shock (34,77).

Lundholm (56) cites Cori and Cori as stating that epinephrine causes the breakdown of muscle glycogen with the production of hexose monophosphate and lactic acid. In his own experiments Lundholm showed that intravenous administration of epinephrine in the rabbit increased the level of lactic acid in the blood.

An aggravating factor is the interference with blood flow in the liver. Lactate is largely metabolized in this organ. Reduction in hepatic blood flow thus helps to keep lactate in the blood at high levels.

Huckabee (41) distinguishes two kinds of lactate production:

- a) due to elevation of pyruvate levels
- b) due to inadequate oxygenation of DPN in body cells.

Only b) occurs in the hypoxia of low perfusion states and it can be proved by simultaneous estimation of lactate and pyruvate levels. In b) pyruvate is not elevated. Blood transfusions and oxygen administration are beneficial in acidosis. Catecholamines increase its severity. Huckabee further states that in experimental shock by slow bleeding

in dogs acidosis (due to excess lactate accumulation) precedes the deterioration of circulatory parameters. Autonomic blocking drugs reduce the excess lactate in the blood even if they lower the blood pressure further.

It thus seems that acidosis is a consequence, rather than a cause, of the hypotensive state.

Weil and Miller (108,110) emphasize the value of the arterial pH as a measure of the severity of shock and an indication of the prognosis.

Peretz and his colleagues (77) advocate the monitoring of blood lactate levels in shock, both for judging its severity and as a prognostic measure. They state that a rise in blood lactate may precede the hypotension. In their series of clinical patients the survival rate was significantly higher in those with initially lower lactate levels. Levels above 8.9 milliequivalents per litre (normal human lactate level at rest: 1.33 milliequivalents per litre or 12 milligrams per 100 ml.) indicate a grave prognosis.

Clinical improvement and lesser vasopressor requirements were invariably accompanied by decreasing lactate levels.

The role of vasoconstriction in tissue hypoxia and excessive lactate production is well illustrated by the experiments of Freeman and his group (23) who found that in hemorrhagic shock normal dogs with vasoconstriction exhibited marked acidosis, while only mild acidosis was evident in dogs which had had a total sympathectomy.

Infusion of alkalis to correct the acidosis of endotoxin shock is reported to improve the pH but have no beneficial effect on survival (25,34,59). Peretz (77) states that bicarbonate administration is of value only when the circulation is improved at the same time. Others (5) claim that infusion of an alkaline solution in acidosis due to shock may in fact be harmful, since it reduces the ventilatory drive and aggravates the hypoxia.

Plasma volume changes.

"Endotoxin shock is not normovolemic shock; rather it is another form of hypovolemic shock", states Lillehei (54).

Gilbert (25) concurs that lowering of the plasma volume may play a contributory part in the late stages of the endotoxin reaction. He reasons that the vomiting and diarrhoea, the oedema of the intestine, the oedema of the abdominal wall and increase in the amount of peritoneal fluid in dogs with peritonitis all tend to bring about a reduction in plasma volume. Measurements of plasma volume in experimental endotoxin shock show reduction. In the experiments of Lillehei and MacLean the average plasma loss was 34.8 ± 13 per cent (52). Coincidentally with the loss of plasma volume the haematocrit increases. Gilbert (25) cites the results of Penner and Bernheim who observed a rise in average haematocrit from 38 to 56 in six hours after endotoxin.

Doberneck and his colleagues (13), using the radioactive I^{131} - labelled human serum albumin (RISA) method, showed

only minimal alterations in blood volume in endotoxin-shocked dogs. There was no difference in the results between the survivors and the dogs that died.

Major blood volume changes are not necessary for clinical septic shock to occur (57). Altemeier and Cole (3) reported that only 25% of their septic shock patients showed haemoconcentration, and that pulmonary oedema was the commonest finding at autopsy in patients who had died. This, they state, is the result of overloading the circulation by transfusion. They state that large amounts of fluid replacement are not indicated. MacLean (57) has noted an adverse clinical response in septic shock patients after multiple blood transfusions, in contrast with patients in traumatic shock who usually respond well.

The role of the capacitance vessels in capillary pooling.

The capacitance vessels are muscular venules and veins which react to sympathetic stimulation by contraction. The diminution in the capacity of these veins is reflected in an increased venous return initially. There is evidence that the muscular venules and veins are sympathetically innervated (20) and that they participate in pressor responses as do the pre-capillary resistance vessels (1). Mellander (63) has shown that capacitance vessels respond with maximum effect to a lower rate of stimulation than resistance vessels. Unlike resistance vessels, they are not sensitive to the effects of

vasodilator metabolites, and remain contracted even when the resistance vessels have dilated under this influence. Lillehei and his group (51) believe that this aspect of capacitance vessel behaviour is instrumental in the trapping of blood in the visceral capillaries in the late phase of endotoxin shock.

Methods of treatment, experimental and clinical.

The diverse concepts of the pathogenesis of endotoxin shock are echoed in the many methods of treatment employed. The existence of multiple treatment methods for a particular condition is usually an indication that not one of them is wholly effective. This is also true for endotoxin shock.

Blood volume replacement alone is not considered effective in endotoxin shock. It will elevate the blood pressure temporarily but does not improve the mortality rate.

Low molecular weight dextran has been shown to return peripheral resistance to less than control levels. Concomitantly, cardiac output, plasma volume and visceral flows rise higher than normal during infusion. Despite this, only 5 per cent of the dogs survived, having been given 2.5 - 7.5 grams per kilogram of low molecular weight dextran. In most instances death was due to a bleeding diathesis. Fibrinogen disappeared from the blood in these dogs.

Dextran, on the other hand, while it does not alter the clotting mechanism, may embarrass the microcirculation by excessive rouleaux formation.

Plasma and saline are both beneficial, but watch must be kept on the right atrial pressure to prevent circulatory overloading. Survival in dogs when either is used alone is less than 10 per cent.

Lillehei at present favours the use of plasma for clinical

volume replacement in endotoxin shock (54). He also points out that the central venous pressure is just as good a guide to adequacy of blood volume as other laboratory methods, and should be monitored in patients receiving blood volume replacement in shock.

The use of antibiotics is favoured, as soon as the diagnosis is made, without waiting for the result of blood culture (3,51,59,92). A combination of antibiotics is advised by Shubin and Weil (92). They recommend intravenous chloramphenicol and streptomycin and tetracycline in addition if the patient has not had it before. Lillehei and his colleagues (54) recommend the combination of Chloramphenicol, Tetracycline and Penicillin, to be given intravenously.

Review of antibiotic therapy when blood culture results are available, is advised by all.

The subject of perhaps the greatest controversy in the context of endotoxin shock is the use of vasopressor drugs in treatment. It will be remembered from the foregoing that opinions are divided on whether peripheral resistance is increased or not in endotoxin shock. This divergence of opinion is carried over to the field of treatment, and it is difficult to find anyone in the literature who condones the use of both vasopressor and vasodilator drugs. Surgeons, it seems, belong either in the vasopressor or the vasodilator camp, but not both.

Altemeier and Cole (3) advocate the use of arterenol

administered in an intravenous drip at a rate sufficient to keep the blood pressure at 80 mm. Hg. or above. It is continued until the blood pressure level remains stable without it. They call attention to the danger of pulmonary oedema in the patient and advocate only moderate blood volume replacement to prevent this complication. Adequacy of fluid and arterenol therapy can also be measured, according to these authors by the urine output, monitored with an indwelling catheter. The hourly urine output should be 25 - 75 mls.

MacLean (59) and Hall and Gold (32) also advocate the use of vasopressors and claim that with their use mortality is reduced. To those who decry vasopressor therapy MacLean (57) replies that patients have been maintained on vasopressors for weeks without ill effects.

Lansing (46) also considers vasoconstrictors the drugs of choice. He feels, as does MacLean (57), that these drugs are indicated to restore the balance between the steadily increasing vasodilatation due to histamine and the steadily decreasing vasoconstriction due to the failure of catecholamine production. By increasing aortic blood flow these drugs maintain the coronary circulation and by a direct action on the myocardium they increase cardiac output.

Wilson (115) suggests that catecholamines should be considered as myocardial stimulants, rather than vasoconstrictor agents.

Moyer and his group (66) stress the importance of blood

volume replacement in combination with norepinephrine.

MacLean (58) has stated that he now favours the use of isoproterenol (Isuprel) in endotoxin shock. This is a sympathomimetic drug which lowers the blood pressure temporarily, due to peripheral vasodilatation. Its action on the heart is similar to that of epinephrine: the rate and the stroke volume are increased with consequent rise in the cardiac output.

Rosenberg and his colleagues (82) are emphatic that vasopressors are harmful, since they exaggerate an already deleterious sympathetic influence. They cite the words of Freeman on the dangers of excessive sympathetic activity: "the very mechanism by which the organism strives to survive, brings about its ultimate dissolution."

Nickerson (68) warns against the use of pressor drugs and against too much preoccupation with the blood pressure. He states: "the blood pressure is a significant determination only when it is related to the condition of the peripheral vascular bed. A normal or elevated pressure operating in the face of severe vasoconstriction may actually induce less blood flow than a much lower pressure associated with peripheral vasodilatation. It is the blood flow, rather than the blood pressure, which maintains the viability of tissues." - and - "vasoconstriction peripheral to the point of measurement may produce stasis in the face of normal or elevated pressure." Nickerson cites von Euler and Remington et. al. who have found that cardiac output is reduced when

vasopressor agents are administered to animals in shock.

Lillehei quotes experimental evidence (48,52,53) that vasopressors given to endotoxin-shocked dogs accelerate death of the animals. He also reports an apparent synergy between endotoxin and metaraminol.

Lillehei and his group (51,54) and Nickerson (73), recently again reaffirmed their stand against vasopressor therapy in endotoxin shock.

In 1948 Wigger and his colleagues (113) reported that pretreatment with the adrenergic blocking agent, dibenamine greatly reduced the incidence of irreversible shock after haemorrhage. In 1950, Remington and his group (80) showed that after dibenamine pretreatment, the animal can survive pressure and flow levels fatal to controls.

The use of dibenamine was later abandoned in experimental work in favour of dibenzylamine (phenoxybenzamine) which is 6 - 8 times more active (27). Both drugs belong to the group of beta-haloalkylamines. They produce blockade of the alpha receptors in blood vessels. The blockade is highly specific, of long duration (24-48 hours) and effective even against strong adrenergic stimuli. They have a slow onset of action due to time required to form ethylenimonium intermediates. These intermediates then form covalent bonds with important biological groups (sulphydryl, amino carboxyl) and this accounts for their long duration of action. According to Goodman and Gilman the peak

effect is reached 1 - 2 hours after intravenous administration of dibenamine, while with dibenzylene it is a little faster. Dibenzylene, unlike dibenamine, has a marked antihistaminic effect. Its blockade of histamine is considered as persistent and as stable as that of adrenergic stimuli.

In experimental canine endotoxin shock pretreatment with dibenzylene has been shown to have a protective effect (28,51,52,59,74,75). The primary drop in blood pressure was still seen, but the secondary decline in pressure was mild. By four hours after endotoxin blood pressure had returned to pre-experiment levels. There was little change in plasma volume or haematocrit. No bloody diarrhoea was seen. At sacrifice the only abnormal finding was liver congestion (52).

Iampietro and his associates (42) found that dibenzylene pretreatment decreased pooling of blood in the liver, but that the arterial blood pressure still fell to the level seen in controls.

It is a curious facet of the literature of endotoxin shock, that while adrenergic blocking agents such as dibenzylene are known to confer a high degree of protection when used in the form of pretreatment, their use after the onset of shock is not nearly so effective (9,53,57,97,109). In fact they have been shown to become less effective as shock progresses (25,72).

Vick, Lafave and MacLean (103), however, obtained favourable

results when they administered dibenzylene to dogs 60 - 180 minutes after endotoxin. They noted dramatic reversal of the depressed renal plasma flow, glomerular filtration rate and urine output even while the blood pressure was at shock levels. The blood pressure, the blood pH and the haematocrit all showed a favourable response. Survival was increased.

Clein and Couves (10) on the other hand found that dibenzylene post-treatment in dogs did not contribute to survival. Weil and Miller (108) found that the use of dibenzylene after shock accelerated the fatal outcome in the dog.

Lillehei and his group (51) report that when dibenzylene alone was given to dogs 30 - 60 minutes after endotoxin, no benefit was obtained. When plasma was infused in addition, the mean blood pressure remained at less than half its normal level, but flow in the superior mesenteric artery increased to exceed the pre-endotoxin level. Lillehei is of the opinion that since adrenergic blocking agents increase the capacity of the vascular bed by at least 15 per cent, fluids must be given in addition to the drug (49). The need for volume replacement in addition to dibenzylene is also stated by others (73,103).

It is postulated that while vasoconstriction or the infusion of a sympathomimetic amine reduce the intravascular volume (22,53), blockade of vasomotor activity results in its prompt increase (42,73).

Dibenzylene is further considered to provide a rapid estimate of the adequacy of the circulating blood volume. When even a large dose of a blocking agent is administered to a normovolemic subject in the recumbent position, little change in blood pressure results. If the subject is hypovolemic the blood pressure shows a profound drop. The amount of volume required to restore the blood pressure provides a semiquantitative measure of the volume deficit (73,75).

Nickerson also claims that dibenzylene is effective in opening up the microcirculation. After dibenzylene the venous haematocrit falls more markedly than the whole body haematocrit. The full significance of this finding in relation to shock is not known, but Nickerson maintains that it indicates a considerable opening of the microcirculation, with decreased plasma skimming and equalized rates of passage of red cells and plasma (75).

Dibenzylene is also said to be of value in the prevention or abolition of pulmonary oedema due to overloading of the circulation by transfusions. In shock, vasoconstriction shifts blood into the pulmonary vascular bed. Inhibition of vasoconstriction can reverse this process. Nickerson cites the case of a patient who developed pulmonary congestion after the slow infusion of only one unit of blood. After the administration of dibenzylene four units could be given rapidly, with clearing of the lungs during the period

of blood administration (75).

It has also been stated that a considerable number of patients in haemorrhagic shock, who failed to respond to blood volume replacement, began to improve 1 - 4 hours after the administration of dibenzylene and that others, who failed to maintain improvement induced by one or more large transfusions, did so after the administration of the blocking agent (70).

One of the undesirable effects of dibenzylene is epinephrine reversal, a dilator action. It is probably due to blockade of adrenergic vasoconstrictor response and consequent unmasking of the adrenergic vasodilator action (27). Green and his colleagues (29) describe the ideal blocking drug as one which blocks the constrictor and dilator responses equally, does not cause epinephrine reversal, and therefore has less tendency to cause hypotension. Green and his group carried out flow studies in dog muscle after dibenzylene and phentolamine administration. These drugs abolished constrictor responses to both norepinephrine and to sympathetic nerve stimulation at L5. With dibenzylene, epinephrine and norepinephrine reversal was seen. The authors hypothesized that the mechanism of the reversal effect may be breakdown of muscle glycogen to lactic acid with fall in blood pH and consequent vasodilation.

Nickerson (69) points out the value of clinical signs when dibenzylene therapy is contemplated. Slow capillary

filling, narrow pulse pressure, cold, sweating limbs and other evidence of vasoconstriction are signs in favour of use of the drug.

The use of phentolamine in experimental endotoxin shock has also been reported (29,47,108). Phentolamine is an adrenergic blocking agent with the same site of action as dibenzylene. In addition it has a weak vasodilator activity (16). Its action is rapid in onset but transient in duration. It produces peripheral vascular relaxation. The blood pressure falls after phentolamine administration. Tachycardia is usual, but undue tachycardia is a sign of overdosage. It stimulates the myocardium, dilates the coronary arteries and increases the stroke volume. In dogs it is reported to increase the systemic arterial pressure, probably because the increase in cardiac output more than compensates for the peripheral vasodilatation (27).

The ability of phentolamine to block the effects of circulating catecholamines was shown by Gillenwater (26), and Kirpekar and his group (44).

Levy, North and Wells (47) reported that pretreatment of rats with phentolamine increased survival in traumatic shock.

Weil and Miller (108) found that phentolamine accelerated death when given to dogs after the onset of endotoxin shock. While there is no standard dose of this drug for the dog, their dosage of 10 milligrams per kilogram would appear to be unduly high.

A large body of opinion (51,54,57,97) supports the claim that there is no physiological lack of adrenal cortical secretion even in the late phase of endotoxin shock.

Nevertheless, Lillehei and his associates (51,52,53,54) have shown that in experimental canine endotoxin shock hydrocortisone pretreatment confers a significant degree of protection. These workers have also demonstrated the value of hydrocortisone when used after endotoxin. The efficacy of hydrocortisone in shock has also been reported by others (31). Even better results were obtained by Lillehei (51) when plasma was given in addition. To produce benefit hydrocortisone has to be given in pharmacological doses, that is, the human patient should receive 1 gram intravenously as soon as the diagnosis is made. This dose may be repeated in 4 - 8 hours. Therapy may be stopped abruptly without tapering the dosage. For use in the dog, a dose of 50 mg. per kilogram is advised.

Shubin and Weil (92) recommend hydrocortisone in a dose of 1 gram per day, or prednisolone, 200 milligrams at the start, followed by 100 milligrams every 6 hours or dexamethasone, 40 milligrams at the beginning of treatment, then 20 milligrams every 6 hours. They also advocate abrupt cessation of steroid therapy on recovery from shock.

The precise mode of action of hydrocortisone is not known. However, Lillehei (51) states that in pharmacological doses

it prevents increase in peripheral resistance, preserves blood flow to the bowel and prevents plasma loss. It has the advantage over dibenzylene that it does not induce hypotension.

Altemeier and Cole (3) recommend the use of steroids only when blood pressure is not restored following the use of vasopressors and blood volume replacement.

Combination of steroids with pressor drugs has been used with success in endotoxin shock. According to Spink (97) the steroid may sensitize the blood vessel wall to the action of the pressor drug, and less vasopressor is required.

Pretreatment with heparin (34,35) is reported by Hardaway to protect dogs from the lethal effects of endotoxin. It has also been shown to prevent the metabolic acidosis which follows endotoxin administration. He also claims success when fibrinolysin is used in established shock.

Surgical intervention, when feasible, to eliminate infected foci is advocated by all recent authors on the subject (3,57,59,97).

The theory has also been advanced (106) that the harmful effects of endotoxin are produced by means of a synergistic effect with kallikrein, a proteolytic enzyme of endogenous origin, which when injected into the experimental animal, reproduces many of the effects of endotoxin. Recent work with the proteolytic enzyme inhibitor Trasylol (FBA) in patients led to clinical improvement and decrease in blood levels of histamine and polypeptide-like agents in 6 of 8 patients (45).

Fine and his group (76) recently reported beneficial effects in man and dog in endotoxin shock by denervation of the abdominal viscera through injection of xylocaine into the coeliac ganglion during shock. Urine flow was resumed, atrial pressure rose, as did the cardiac output and systemic blood pressure.

The induction of tolerance to vasoconstriction by repeated, small injections of norepinephrine has been shown to render experimental animals markedly resistant to the effects of endotoxin (51).

Extracorporeal circulation combined with dibenzylene has been accompanied by some success in experimental endotoxin shock. The use of hypothermia has not been effective (9,52).

Weale (105) considers that the hypotension of shock is due to acute myocardial failure. In his opinion, improvement in the treatment of irreversible shock will depend on the facilitation of oxygen transport to the myocardium. Hyperbaric oxygenation may become a useful means of achieving this end. Extracorporeal circulation should also be considered. In any case, Weale advocates the administration of oxygen by the best available means. Oxygen is also advised by Altemeier and Cole (3).

Douglas and his colleagues (14) recently reported a diagnostic test for the demonstration of endotoxin in the patient's serum. The test is based on the altered reactivity

of blood vessels to epinephrine which results from endotoxin, as described by Thomas (98). The test is conducted as follows: 7 millilitres of the patient's serum are injected intravenously into an albino rabbit. Then at neighbouring sites 50 and 100 micrograms of epinephrine are injected into the skin. A positive test shows haemorrhagic necrosis at the injection sites in the skin.

The purpose of the experiment.

The "raison d'etre" of shock experiments on animals is the need to establish controlled studies in an organism with a physiology akin to that of man, in order to achieve understanding of the pathophysiology of shock and explore effective avenues of treatment.

The difficulty of studying endotoxin shock in patients is pointed out by Udhoji and his colleagues (78): "the subjects are sick persons who have had infection for varying periods of time; previous treatment is also a factor which is entirely uncontrolled. Moreover, septic shock occurs preponderantly in older patients who are much more likely to have degenerative vascular disease with limited coronary and cerebral circulation. During shock patients are often moribund, and this presents formidable technical problems. Therefore conclusions based on such studies necessarily must be viewed with reservation until a sufficient number of studies have been completed to secure statistical validation."

The laboratory animal, however, is not a perfect substitute for man. Zweifach (117) points out that the existence of species differences is taken for granted, but the spread of "normal" values within any species is so great as to make many of these indices almost meaningless by themselves. Furthermore, animals often harbour subclinical infections, which under experimental conditions may become fulminant. Structural differences in different organs may enable them

to serve as blood depots. In the dog and the cat the liver and the spleen can add up to 30 per cent of the blood volume. The differential organ sensitivity to endotoxin is another variant. In man, the kidneys are the target organ, while in the dog it is the intestine and in the rabbit, the lung (51).

Even if we restrict our experiments to one animal species we may find it difficult to reproduce the clinical picture. For humanitarian reasons, and for experimental convenience, the use of anaesthesia is necessary. There is evidence that anaesthesia may significantly alter certain parameters. This is in contrast with the human patient who is usually awake and unanaesthetized.

Price (78) reports that anaesthetics, such as thiopentone, potentiate the vasoconstrictor effects of catecholamines. This may be of physiological significance, since it would cause blood vessels to constrict in response to physiologically normal levels of circulating tissue catecholamines. Price also states that thiopentone suppresses vasomotion and causes plethora of the capillary bed.

The hypoventilation associated with anaesthesia results in reduced elimination of CO_2 . Since CO_2 is a potent stimulant of the sympatho-adrenal system, catecholamines are mobilized as a result (15,78).

Others also claim that anaesthesia exerts an adverse effect on the experimental animal in shock (48,52,87).

Moyer (65) has shown that norepinephrine infusion sufficient to raise the blood pressure by 20 mm. Hg. or more and maintain it at this level, will result in death of the animal in 18 hours if the animal is anaesthetized. This is not so with the unanaesthetized animal.

Sjostrand (96) feels it is unjustifiable to draw conclusions regarding the general regulation of circulation in human beings from results obtained in experiments under anaesthesia, in view of the marked haemodynamic disturbance initiated by anaesthetic agents.

Another likely divergence from the clinical situation is the universally employed method of administration of endotoxin in a single, massive dose. Udhoji, Weil and their colleagues (102) point out that this probably does not simulate the conditions which exist during the development of shock in man.

The purpose of this study was threefold:

Because the endotoxin-shocked man is usually awake and unanaesthetized -

1. to evaluate the effect of anaesthesia on the endotoxin-shocked animal.

Because endotoxaemia in man occurs continuously over a relatively long period of time -

2. to evaluate the differences between rapid injection of endotoxin and its infusion over a period of two hours.

Because pretreatment with dibenzylene protects the animal from the lethal effects of endotoxin, while treatment after endotoxin is far less effective -

3. to test the value of simultaneous administration of phentolamine, an adrenergic blocking agent of rapid onset, but short duration of action. This approach was prompted by the reports that even after intravenous administration of dibenzylene a delay is incurred before its action is exerted (27,73,75). It was planned to secure rapid, early adrenergic blockade with phentolamine while waiting for dibenzylene to develop its sustained blockade. In the treatment of an emergency situation such as shock, the elimination of delay in drug action would seem warranted.

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CHAPTER II

METHOD AND MATERIALS

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M E T H O D A N D M A T E R I A L S .

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M E T H O D

1. Animal care.

This study is based on seventy-two experiments. In each experiment a healthy adult mongrel dog was used as subject. For at least two weeks prior to the experiment every dog was kept in the Vivarium under veterinary care. This ensured that the dogs were in optimum condition when used for study.

2. Plan of Study.

The experiments were divided into two principal groups:

- a) experiments on unanaesthetized dogs - U.
- b) experiments on anaesthetized dogs - A.

Each of these groups was further subdivided into four sub-groups:

- (i) endotoxin infusion, no treatment - E.
- (ii) endotoxin by rapid injection, no treatment - E(S).
- (iii) treatment only, no endotoxin - T.
- (iv) endotoxin infusion and treatment -ET.

The number of experiments in each group is represented in the table:

U				A			
E	E(S)	T	ET	E	E(S)	T	ET
8	4	4	8	15	4	14	15

3. Preparation for Experiment.

(1) pre-operative.

The dog was allowed water but no food during the 24 hours preceding the experiment. The quarters in which the animals were kept permitted a liberal amount of exercise.

The weight of the animal was recorded immediately before surgery.

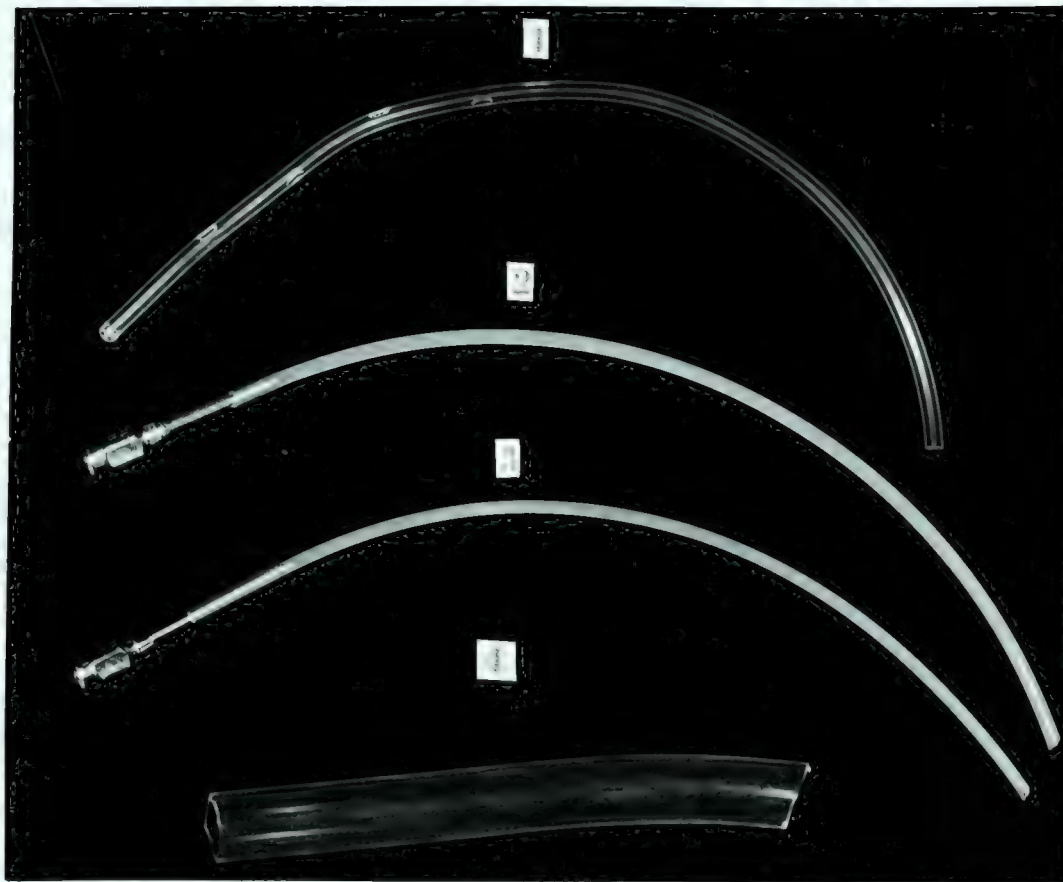
Anaesthesia. All dogs received 30 mg. per Kg. Pentobarbital Sodium (Abbott) intravenously. Dogs in the unanaesthetized (U) group, operated upon on and after 7th February, 1964, were given Thiopental Sodium (Abbott) for injection, U.S.P. intravenously, in a dose of 25 mg. per Kg. body weight. The reason for this change will be discussed below.

Skin preparation. After the induction of anaesthesia, regions of the skin in which incisions were to be made were shaved thoroughly with an electric shaver. The skin was then washed with liquid surgical soap, painted with iodine, and draped with sterile towels.

(2) operative.

Catheterization of bladder. Early experience with soiled towels showed that catheterization was best carried out before making the skin incisions. After initial failures with the available Foley catheters, no difficulty was encountered when 14 or 16 French Stomach tubes (Levin type, 50" long) (Becton, Dickinson and Co., Rutherford, N. J.) were used for the purpose. Fig. 1.(1).

Fig. 1.



1. urethral catheter
2. Size P.280 polyethylene cannula
3. Size P.260 polyethylene cannula
4. tracheostomy tubing.

Tracheostomy was performed through a 1 1/2" transverse incision in the anterior aspect of the neck, midway between the lower end of the larynx and the suprasternal notch. "Tygon" tubing of 3/8" or 1/2" internal diameter was used. Fig. 1 (4).

In dogs being prepared for the unanaesthetized experiment, the tracheostomy tube was secured to the trachea by a single 2-0 silk suture. It was easily removable later by cutting the suture inside the tube.

Cannulation of blood vessels. The vessels cannulated and the purpose for cannulation in each case were as follows:

- (a) right vertebral artery - for measurement of blood pressure and for collection of arterial blood samples. The tip of the cannula was advanced to lie in the aorta.
- (b) right external jugular vein, for measurement of central venous pressure and for collection of venous blood samples. The tip of the cannula was passed to the superior vena cava. (a) and (b) were exposed through a 2" longitudinal incision in the right lower anterior aspect of the neck, midway between the lateral border of the trachea and the lateral neck margin, above the first rib.
- (c) left external jugular vein through an incision in the left side of the neck symmetrical in position with that used for (a) and (b). The cannula, passed into the vessel for a distance of 2", served to deliver endotoxin

and the drugs used.

(d) left saphenous vein. The cannula was introduced into this vessel about 2" distal to its opening into the femoral vein, then passed proximally until the tip lay just inside the femoral vein. By occluding venous flow in the femoral vein with a loop of 2-0 silk placed just proximally to the sapheno-femoral junction, the venous return was diverted via the cannula in the saphenous vein to a measuring cylinder held at a constant level. This cannula served to obtain blood for the measurement of venous outflow from the hind limb.

(e) In experiments using the bubble flow meter the right femoral artery was cannulated with two short cannulae, one of which served to deliver blood from the vessel to the flow meter, while the other returned the blood from the flow meter to the femoral artery.

Materials used for cannulation of blood vessels.

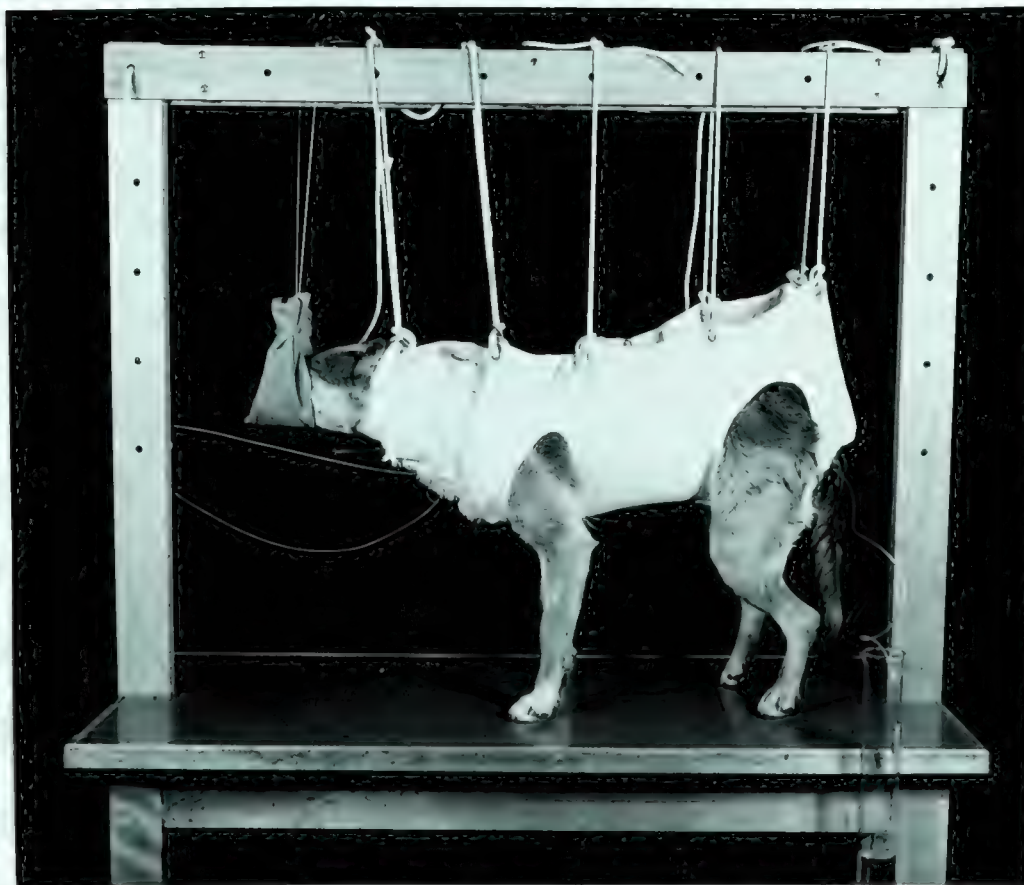
"Intramedic" polyethylene tubing (Clay-Adams, Inc., New York) size 280 was used whenever possible. Very occasionally, in small dogs with small vessels, size 260 or 240 had to be used. While slightly more difficult to introduce, the size 280 cannula gave excellent service and flow unhindered by clotting problems. In view of the firmness of this type of tubing it was important to cut only a shallow bevel in the tip to prevent it from damaging the vessel as it was passed proximally. A No.13 needle with its sharp tip sawn off, in order to prevent damaging

the polyethylene tube, was introduced into the free end of the cannula, to serve for connection with syringes, manometer, etc. Fig. 1 (2,3).

(3) post-operative.

Unanaesthetized animals. Prior to Feb. 7, 1964, dogs intended for the unanaesthetized experiment (U) were prepared in the afternoon of the day preceding the experiment. This method was in vogue in the laboratory when the present study began. Thus, as soon as the operative preparation was completed, the dog was removed from the operating table and placed in a supporting harness suspended from a stand designed for this purpose. (Fig.2)

Fig. 2.



Dog in harness, showing positions of tracheostomy tube, cannulae (a) and (b) and urinary catheter.

In this harness the dog was left overnight and the experiment was carried out the next day. The harness supported the dog until the effects of anaesthesia wore off. Thereafter the dog was able to stand. However, to protect the cannulae and the tracheostomy, the rigging had to be firmly secured, allowing virtually no movement during the night when the dog was unobserved.

A great disadvantage of this harness was the dependent oedema noted in the paws by next morning, which increased in intensity with time. An even greater disadvantage was the fact that in spite of every effort to restrain the dogs in the harness during the "unobserved hours" some dogs did succeed in dislodging their cannulae and tracheostomy tubes, and were found in varying stages of distress by the next morning. These dogs thus failed to qualify as experimental subjects and had to be discarded. Some five or six experiments were forfeited in this way.

Hunger was another undesirable feature of this method of preparation. By the time the experiment began the dog had been without food for 48 hours. Fighting the restraining harness, with its attendant frustrations, also seemed a factor to be avoided.

It appeared, therefore, that even if the dog was intact in the harness by next morning, with hunger, oedema and frustration, unnecessary variables were being introduced into the experiment. Four dogs in the U_E and four dogs

in the U_{ET} groups were used under these conditions. It was therefore decided to discontinue this method of preparation. The change to intravenous thiopentone sodium, 25 mg. per Kg., as mentioned above, gave 20-25 minutes of anaesthesia for the preoperative and operative preparation. Absolute readiness and speed in surgery made this time interval quite sufficient for the task in hand. The dog was transferred to the harness immediately after the operative preparation. The effects of anaesthesia were allowed to wear off. This took 60-90 minutes. The experiment could then commence.

Anaesthetized animals.

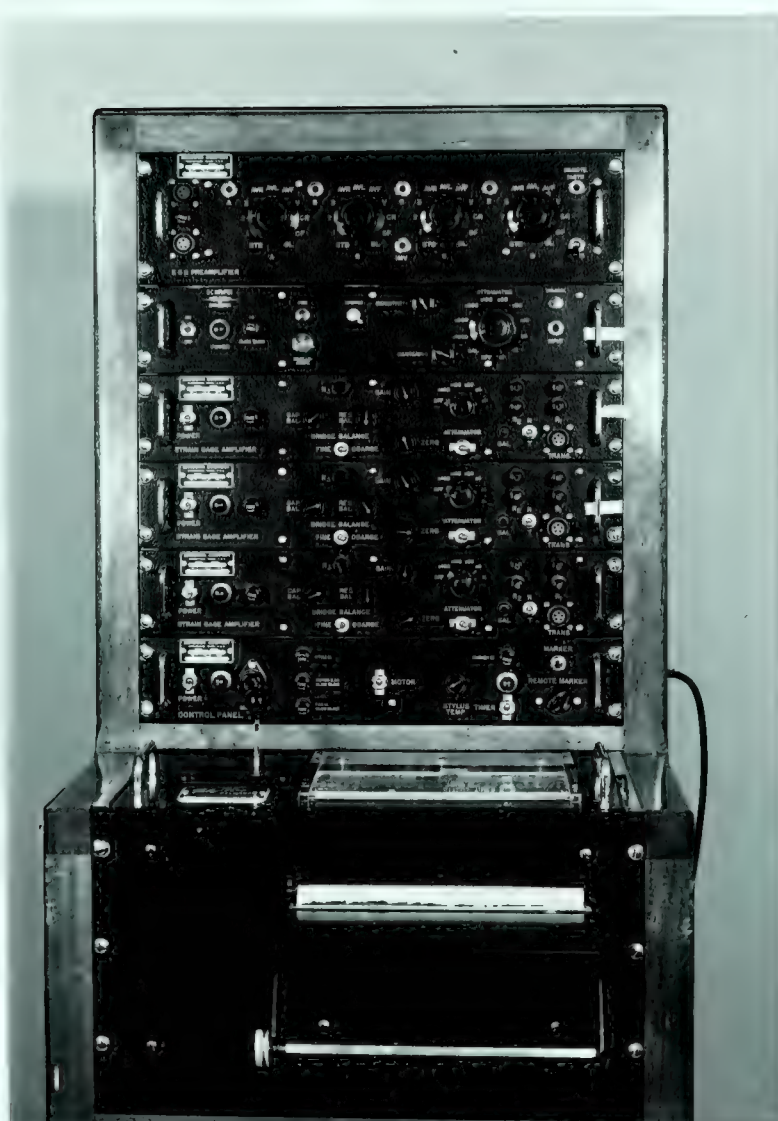
These animals remained on the operating table until the conclusion of the experiment. In the majority of cases no further anaesthesia was required. Occasionally, when a dog showed signs of awakening, 1/2 to 1 ml. (30-60 mgs.) of the nembutal solution was administered through cannula (c) to maintain the animal in a lightly anaesthetized state.

Measurement of parameters.

(a) Blood pressure.

Cannula (b) was connected to a Statham Pressure Transducer, Model P 230 b. (Statham, Hato Rey, Puerto Rico) linked with a Sanborn Poly-Viso Recorder. (Fig. 3).

Fig. 3



The Statham transducer was later replaced with a Sanborn Pressure Transducer, Model 267A (Sanborn Co., Waltham, Mass., U.S.A.). The Wheatstone Bridge of the Sanborn recorder was balanced and the strain gauge calibrated before each experiment. The dog's blood pressure was recorded for at least 30 minutes prior to the start of the experiment, then continuously throughout its duration.

b) Central venous pressure.

Cannula (a) was connected to a glass manometer with its

zero mark at the level of the right atrium.

The central venous pressure (CVP) was monitored continuously for at least 30 minutes before the experiment and throughout its duration. It was recorded in cms. of water.

c) Hind limb flow.

The free end of cannula (d) was held by the assistant over a sterile syringe or measuring cylinder at a predetermined level (a mark was made for this purpose on the leg of the operating table). Blood was collected during a ten second period. To aid accuracy, a five-second countdown was given for the release of the haemostat on the cannula and another five-second countdown for its reapplication.

Initially the procedure was doubled to check accuracy. When accuracy was evident, the double check was abandoned, since it was noted that frequent use of the cannula encouraged clotting within it. Hind limb flow was measured every 30 minutes during the experiment. The blood was returned to the dog unless its volume was less than 2 mls.

d) Urine collection.

The catheter drained directly into a measuring cylinder. The urine output was measured every 15 minutes.

e) Acid-base balance studies.

Arterial blood from cannula (b) and venous blood from

cannula (a) were obtained before the experiment, then every 30 minutes for 2 1/2 hours then every 60 minutes for the remainder of the experiment. 2cc samples of blood were collected anaerobically into a 2.0 or 5.0 cc syringe treated with liquid paraffin and containing 0.2 mls. 1:1000 heparin. The samples were processed according to the Astrup method (93,94). Fig. 4. Information was obtained on arterial pH, venous pH, arterial pCO_2 HCO_3 and total CO_2 levels.

Fig. 4.



Preparation of endotoxin. The endotoxin used in these experiments was Lipopolysaccharide E. coli 0111:B4, prepared by the Difco Company of Detroit in a desiccated form and supplied in vials of 100 mgs. The Boivin trichloroacetic acid procedure, as modified by Webster, Sagin, Landy and Johnson, is employed in the extraction of this endotoxin (107). It is standardized using Webster strain white mice, to give LD₅₀ -S of 0.5 mg. or less. It is further standardized on the basis of its nitrogen and phosphorus content and its capacity to elicit antibodies in rabbits. It is stable for extended periods of time when stored in a cool, dry place. The contents of the vial (100 mgs. endotoxin) were shaken with 5 mls. sterile saline until a fine suspension was obtained. A dose of 5 mgs. endotoxin per Kg. body weight was then removed from the vial and instilled into a bottle containing 250 mls. 5% dextrose in water (or N saline, depending on which solution was available). This volume was large enough to be given in an even drip through a standard intravenous-giving set. All dogs received endotoxin in a 250 ml. infusion, except those in the U_E(S) and A_E(S) groups. These dogs received the dose in a rapid injection, taken from the suspension in the vial.

Preparation of Phentolamine.

10 mgs. of Phentolamine methane-sulphonate (ROGITINE, Ciba) were dissolved in 100 mls. sterile saline. All dogs

received the same dose irrespective of body weight.

Preparation of Phenoxybenzamine.

100 mgs. of Phenoxybenzamine HCl were dissolved in 500 mls. of normal saline, giving a concentration of 0.2 mg. per ml. The dose of dibenzylene was 0.5 mgs. per Kg. body weight, i.e. for a 15 Kg. dog 45 mls. of this solution were given.

4. The Experiment.

With all preparations completed and the dog stable, the experiment was begun.

In groups E and ET the endotoxin was infused at an even rate over a period of two hours (drip rate of 30 drops per minute).

In groups E(S) the endotoxin was rapidly injected.

All dogs received only one dose of endotoxin. The blood pressure was traced continuously for the first thirty minutes; thereafter short tracings were taken every 15 minutes.

The central venous pressure and urine output were recorded every 15 minutes.

Hind limb flow was recorded every 30 minutes.

Arterial and venous blood samples for acid-base balance studies were taken every 30 minutes for the first 2 1/2 hours, then hourly until the conclusion of the experiment.

In treatment control experiments (T) phentolamine and dibenzylene were given together to 4 dogs each in

both the unanaesthetized and anaesthetized groups. The phentolamine was infused over a period of 2 hours, while dibenzylene was given in half that time. In addition, in the A_T group, 2 dogs received phentolamine alone and one dog dibenzylene alone.

In the ET groups treatment was given when a marked fall in mean blood pressure was noted (70 mm. Hg. or more), when acidosis became manifest, when bloody diarrhoea occurred, or after more than 3 hours experiment time. The latter policy was deemed reasonable on the grounds that in the control groups dogs, which had failed to exhibit any of the features mentioned above, often died.

5. Animal care after the experiment.

At the conclusion of the experiment on anaesthetized dogs all the cannulae were removed from the blood vessels. The vessels were ligated and the wounds closed in layers. The urinary catheter was removed. If anaesthesia was light or the dog conscious at the time of leaving the laboratory, the tracheostomy tube was removed also. Otherwise it was removed the next day.

Unanaesthetized dogs had their cannulae tied into a knot just above the skin and the rest cut off. It was deemed preferable to leave the proximal end of the cannulae in situ than to submit the dog to anaesthesia for their removal at a time when it may have adversely

affected survival.

The urinary catheter was removed. The tracheostomy tube was removed if breathing was unobstructed.

All dogs which were alive at the end of the experiment were returned to the post-operative ward of the Vivarium. Here they were placed in individual cages and maintained under optimum conditions of cleanliness and observation. The postoperative progress of these dogs was also followed by the Director of the Vivarium, a veterinary surgeon.

At first, only water was allowed following the experiment. The animal was nursed back to normal diet at a rate consistent with its condition and interest in food.

To eliminate unnecessary variables which may have interfered with survival, no drug of any sort was administered to the dogs other than those mentioned above in connection with the experiment proper.

Dogs which survived for one week following the experiment were regarded as survivors. After this period they were sacrificed.

Tissue specimens were taken from several dogs for histological study.

6. Flow measurements, using bubble flow meter.

The influence of Rogitine and Dibenzylene on hind limb resistance in the absence of endotoxin was studied

by means of a bubble flow meter designed by Nash and Milligan (67). To prevent blood clotting in the apparatus heparinization of the dog prior to the experiment was required. Heparin Sodium, U.S.P. (Riker), 10 mg. per cc., was given in a dose of 2 mg. per Kg. body weight before the experiment. Thereafter 10 mgs. of Heparin were given every hour.

The necessity for heparinization precluded the study of flow changes induced by endotoxin, since heparin pretreatment is known to protect from the effects of endotoxin (33,34,35).

Principle of the bubble flow meter.

Blood diverted from the femoral artery was passed through the flow meter, then returned to the femoral artery. In its passage through the flow meter, a bubble was introduced into the blood and circulated with it through a loop of known volume. By means of a photoelectric cell the time taken for the bubble to traverse the loop could be detected and recorded on the blood pressure tracing. A bubble trap prevented the bubble from entering the femoral artery. With each bubble trapped, a new bubble was automatically introduced, making continuous flow measurement possible.

The flow meter, in progressive stages of assembly is shown in figures 5, 6 and 7.

Figure 5.

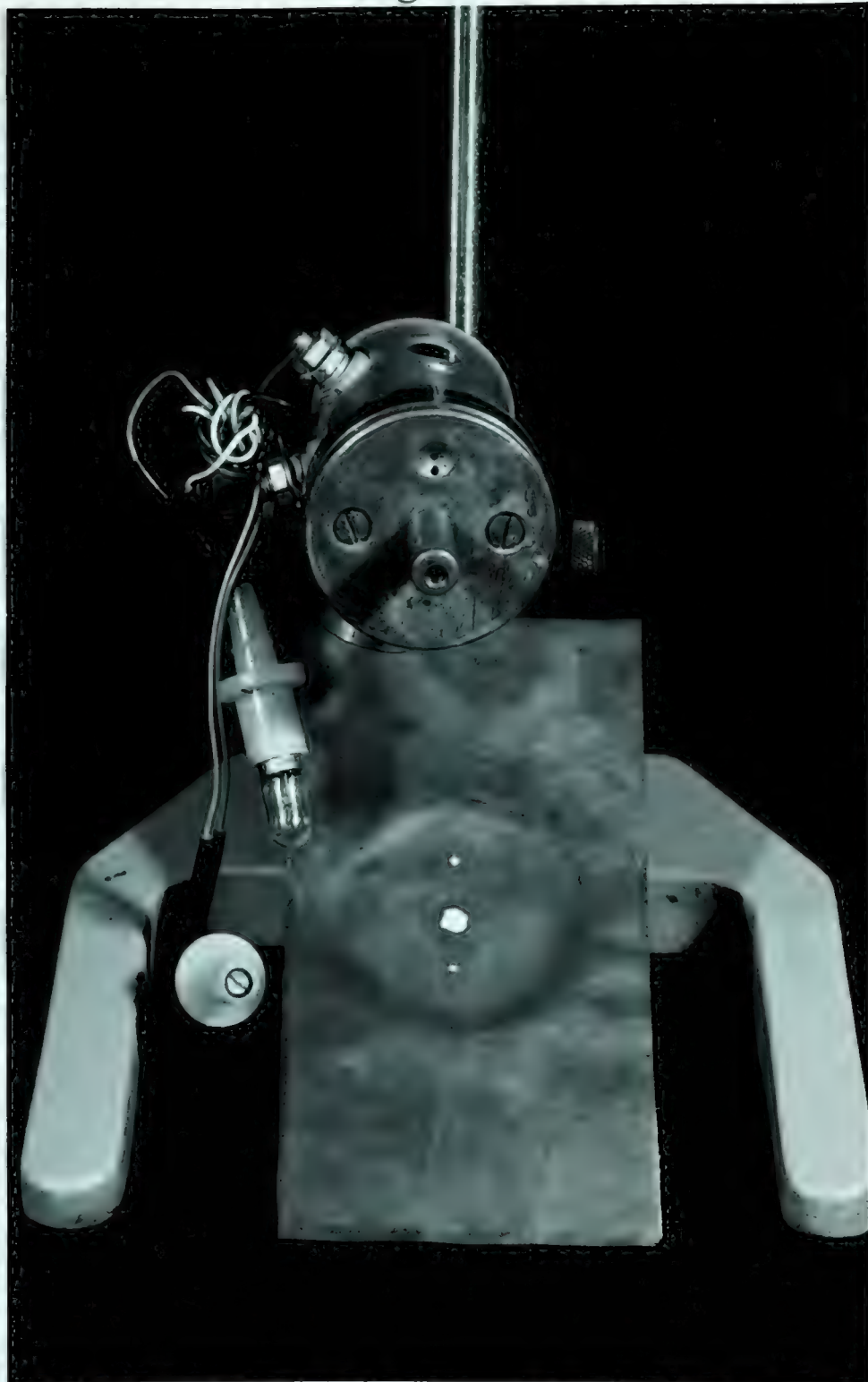


Figure 5 shows the air pump with small apertures above and below its central supporting rod. These apertures serve for the injection and return of air. A rubber diaphragm fashioned out of a sheet of Pure Latex Dental Dam by means of small holes punched in it to coincide with the air injection and air return apertures is seen below the air pump.

Figure 6.

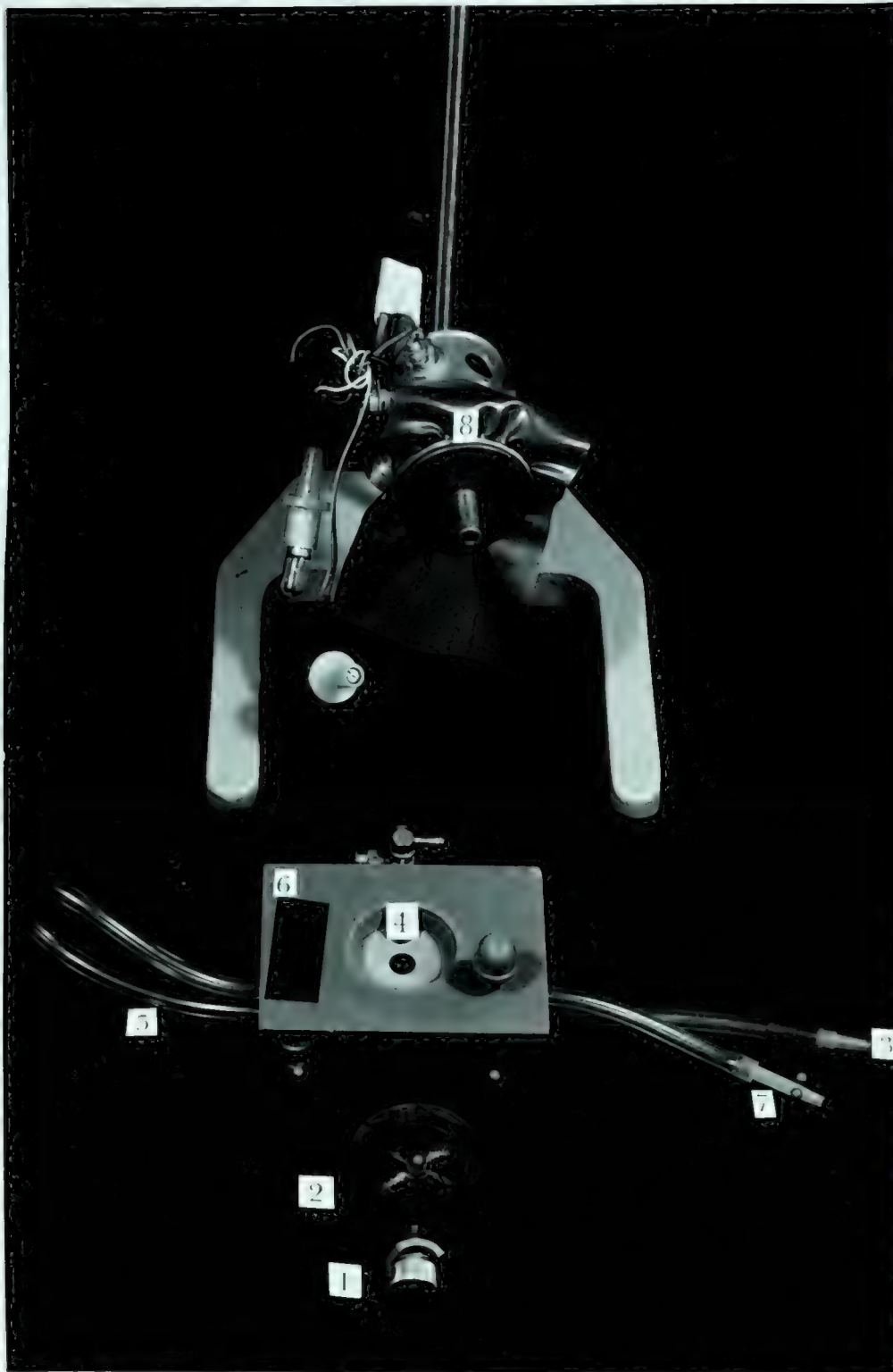


Figure 6 shows the diaphragm (8) in place. A screw (1) serves to hold the transparent Perspex bubble trap cover (2) over the bubble trap (4). Blood enters from the femoral artery via (3) and picks up the bubble from the air injection

valve (in 8). The bubble circulates through the loop (5). Its passage is recorded by the photo-electric cell (6). The impulse thus generated causes the release of a fresh bubble while the previous bubble is caught in the trap (4). The blood returns to the femoral artery through the outlet tube (7).

Figure 7.

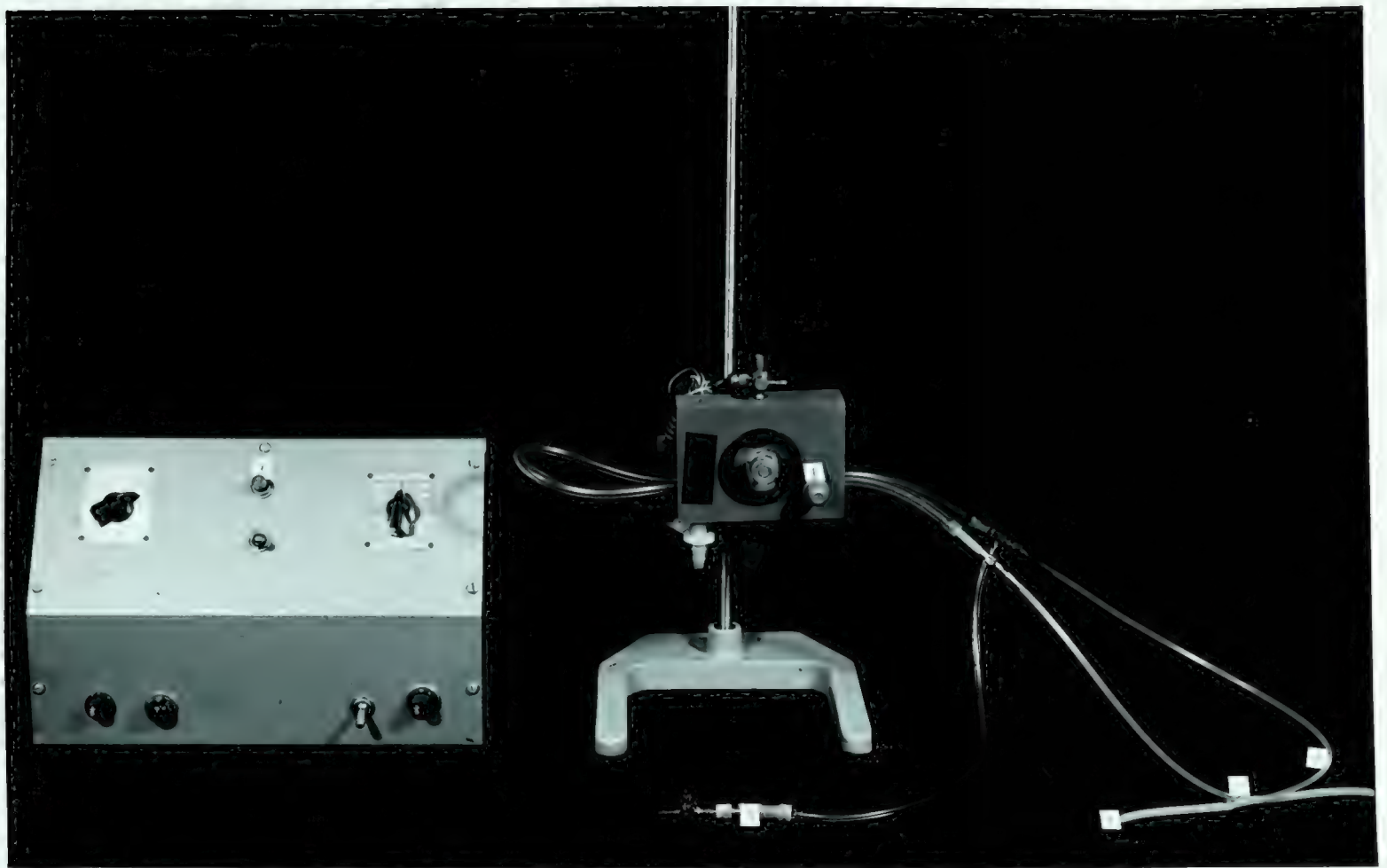


Figure 7 shows the entire apparatus assembled as in an experiment. Blood from a simulated femoral artery (1) is led via a polyethylene tube (2) to the flow meter (4). The connection with the Sanborn pressure recorder is represented by (5). The case (6) houses the flow meter circuit. Blood is returned to the femoral artery via a polyethylene tube (3).

This flow meter has the advantage that it permits simultaneous recording of the arterial blood pressure through a simple connection to the pressure recorder as illustrated above. The passage of the bubble past the photo-electric cell causes a pressure impulse which is superimposed on the blood pressure and is recorded on the pressure tracing.

Calculation of flow.

The volume of the loop through which the bubble is passed was 2.9 mls.

"t" is the interval in seconds between consecutive bubbles, as measured by the timing device of the pressure recording apparatus.

The minute flow, F, through the vessel is then:

$$F = \frac{60}{t} \cdot 2.9 \text{ mls.}$$

Calculation of resistance.

The resistance in the hind limb was calculated using the following formula:

$$R = \frac{\text{A-V pressure difference}}{\text{minute flow}}$$

$$= \text{resistance in peripheral resistance units (30).}$$

The dogs used for the bubble flow meter studies were sacrificed immediately following the experiment.

CHAPTER III

RESULTS AND DISCUSSION

R E S U L T S A N D D I S C U S S I O N

When endotoxins of Gram-negative bacilli are liberated into the bloodstream of man or of an experimental animal, they initiate haemodynamic and metabolic changes which may lead to death. The high mortality associated with endotoxaemia in clinical practice necessitates urgent study of the mode of action of endotoxins in order to secure avenues of effective treatment.

Paradoxically the clinical patient is not the most suitable subject for the study of endotoxin shock. As pointed out by Udhoji and his group (102), observations in a clinical setting are subject to diversity owing to the varying periods of endotoxaemia as well as differences in treatment prior to study. Degenerative vascular disease due to age puts a further bias on the circulatory parameters.

A suitable experimental animal is therefore the logical choice for study of the mode of action of endotoxins. The dog, with its easy availability, convenient size and physiological similarity to man, fills this need. Unlike in man, the study of endotoxin shock in the dog can begin on the basis of a standardized set of circumstances. At the outset of the experiment the parameters studied occupy a position near a common baseline.

In spite of the degree of standardization possible in

experiments on dogs, however, the disadvantage of a major variable factor still remains. This variable is the individual dog used as experimental subject.

The "adult mongrel dogs" used in this study were all treated alike as far as possible for two weeks prior to the experiment. They were well fed and housed, and their health was carefully supervised under the direction of a veterinary surgeon. Yet it cannot be said that any more than a broad comparison could be drawn between any two dogs used in this series. They had a varied background in intermixture of breed, sex, weight, age, nutrition and recent or past disease (e.g. distemper, parasitic infestation).

Simeone (31,95) points out that even within a species, individual differences exist which may affect the results of experiments significantly. According to him "despite..... efforts toward matching pairs of animals for individual experiments, physiological differences of apparent survival value make their appearance early during the course of the experiment." He also states that the time of the year at which the experiment is performed is of significance, since animals withstand loss of blood and hypotension better in winter. Even the time of day at which the experiment is carried out may influence the results.

The individual susceptibility of dogs to endotoxin is also pointed out by Cavanagh and his associates (8).

Another factor modifying the animal's response to endotoxin may be the influence of pre-experiment animal care on the

properdin titre of the dog. The concept of properdin was introduced by Pillemer in 1954 (19). Properdin is a plasma globulin which in the presence of a critical concentration of magnesium ion activates the C^3 component of complement to lyse extracellular bacteria. This is due to the affinity of properdin for certain polysaccharides in bacterial cell walls, chiefly of the Gram-negative flora. A low properdin titre is associated with a low natural resistance to infection. Malnutrition results in a low titre. The titre is proportional to the degree of animal care. It is quickly restored to normal with sound nutrition and the elimination of disease.

It appears, therefore, that even though the dog is a suitable subject for this experiment, shock studies in the dog still have certain limitations. These we must accept. The disadvantages are balanced, at least in part, by several factors.

If it is apparent that even within a species, such as the dog, differences exist between individuals which may influence experimental results, it is all the more important to design the experiment in a manner which will closely parallel the clinical situation.

With this aim in mind it was therefore decided to administer endotoxin to dogs by a slow infusion lasting two hours which is probably a closer approximation to the development of

endotoxaemia in man than the rapid injection of the same dose of endotoxin. This was a departure from the conventional methods of study reported in the literature so far. In view of the fact that the pathophysiology of endotoxin shock following a rapid injection of endotoxin is well documented, in this study only a limited number of dogs were given the rapid injection.

The use of anaesthesia in experimental endotoxin shock in dogs is a deviation from the clinical parallel, since the patient with this condition is usually awake and unanaesthetized. As outlined in the Introduction, it has been argued that shock experiments on anaesthetized dogs are of no value, since the use of anaesthetic agents alters the reactivity of blood vessels. To the animal researcher, however, shock experiments on unanaesthetized dogs present considerable difficulty. It seemed necessary, therefore, to examine the problem from both angles. Thus, both anaesthetized and unanaesthetized dogs were subjected to endotoxin shock to evaluate the influence of anaesthesia on the experiment.

The varied methods of treatment used in experimental and clinical endotoxin shock were outlined in the Introduction. Evidence was presented to the effect that endotoxaemia is attended by a marked sympathomimetic response either because endotoxin is a sympathomimetic substance itself, or because it sensitizes the blood vessels to endogenous sympathomimetic agents. The deleterious effects of prolonged, severe vaso-

constriction were discussed. In this context reference was made to the use of adrenergic blocking agents. It is known that dibenzylene pretreatment protects the animal from the lethal effects of endotoxin while dibenzylene used after shock has ensued no protective effect. It has also been reported that a delay is incurred before dibenzylene exerts its full sympathetic blockade. Since shock in clinical practice is an emergency situation, delay in the onset of action of dibenzylene is regrettable. For this reason phentolamine was given in addition to dibenzylene to dogs receiving treatment, with the aim of producing rapid adrenergic blockade from the outset, while waiting for dibenzylene to develop its sustained blockade. No other form of treatment was given.

The rapid injection v. the slow infusion of endotoxin.

Sudden, massive endotoxaemia probably does not occur in man. Endotoxaemia is more likely to develop gradually, over the course of a few hours. For this reason, the universally documented method of producing experimental endotoxaemia by a rapid injection of endotoxin is unlikely to represent faithfully the clinical picture in man. In the following figures a comparison is shown between the effects of endotoxin administration to dogs by slow infusion and by rapid injection.

The letter code is interpreted as follows:

U refers to experiments on unanaesthetized dogs.

A refers to experiments on anaesthetized dogs.

The suffix E represents endotoxin infusion over 2 hours.

The suffix E(s) represents rapid injection of endotoxin.

Examples.

A_E represents endotoxin infusion in anaesthetized dogs.

$U_E(s)$ represents rapid injection of endotoxin in unanaesthetized dogs.

There is a well-documented difference in the behaviour of the blood pressure between man and dog in endotoxaemia. In man the blood pressure is said to decline gradually (82,103) while in the dog a precipitous fall in the blood pressure is observed following endotoxin injection, with partial recovery to near-normal levels, followed by a terminal decline (52,53). Since endotoxaemia in man is not likely to supervene with the suddenness of a massive injection as in the experimental animal, the comparison is not entirely valid. Slow infusion of endotoxin in this study showed that gradually developing endotoxaemia in the dog produces a gradual decline in the mean blood pressure, similar to that described in man. While the initial sudden drop in pressure is not seen, the decline at the end of two hours is virtually identical with that produced by rapid injection. (Figs 8 and 9).

Further it is interesting to observe that marked fall in the mean blood pressure occurs during the infusion of

Figure 8.

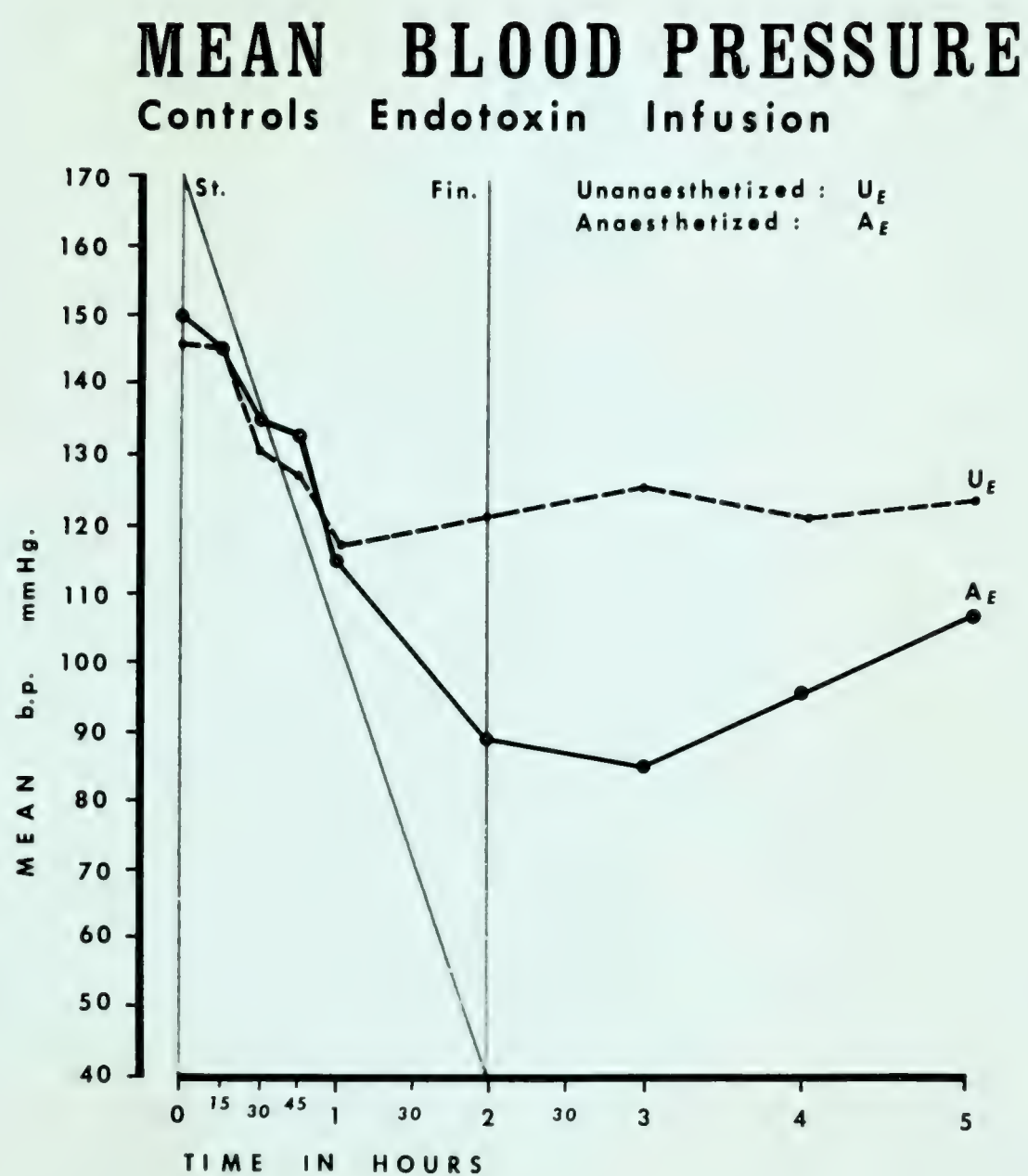
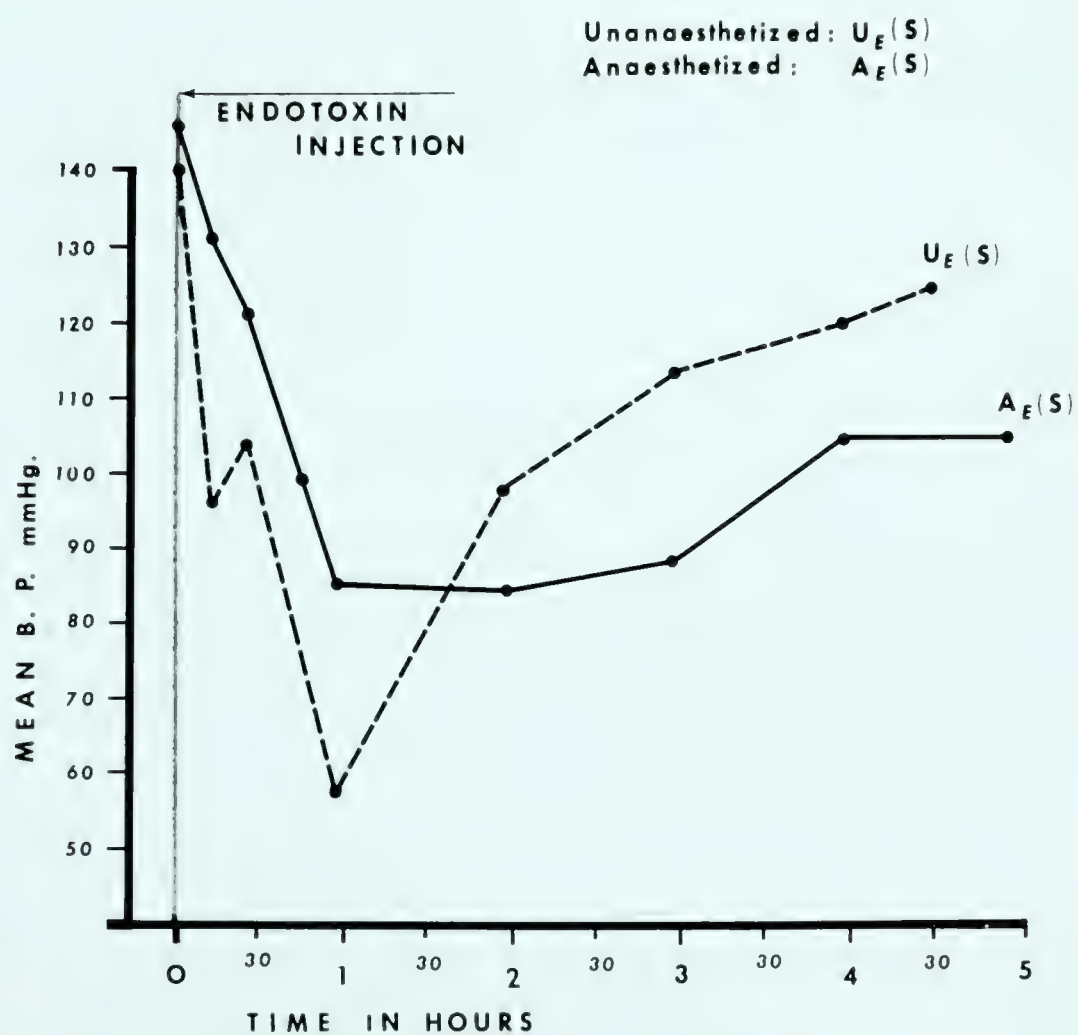


Figure 9.

MEAN BLOOD PRESSURE

ENDOTOXIN CONTROLS

(Rapid Injection)



endotoxin at a time when only a fraction of the endotoxin dose has been administered.

The pulse pressure, likewise, exhibits a steady downward trend in the slow infusion group which is maintained for a while even after the infusion is discontinued. This is in contrast with the group receiving the rapid injection, in which after the initial drastic fall and subsequent fluctuations the pulse pressure recovers to near normal levels. (Figs 10 and 11).

Both groups show a fall in central venous pressure. Again, in the slow infusion group a steady, downward trend is seen over five hours, while after the initial fall it fluctuates in the rapid injection group. (Figs 12 and 13).

With only half the endotoxin dose administered (at one hour) by slow infusion, the reduction in hind limb venous outflow is equivalent to that following rapid injection of the full dose. (Fig. 14).

An interesting observation in the slow infusion group was the tendency to diuresis during the second hour of infusion. The diuretic response was noted to be considerably more marked in the dogs which eventually became survivors. (Fig. 15).

The mechanism of this diuresis is not entirely clear. It is tempting to hypothesize that after initial suppression of renal blood flow reactive hyperaemia develops in the kidneys, accounting for the increase in the output of urine.

Gilbert (25) states that after endotoxin, resistance in

Figure 10.

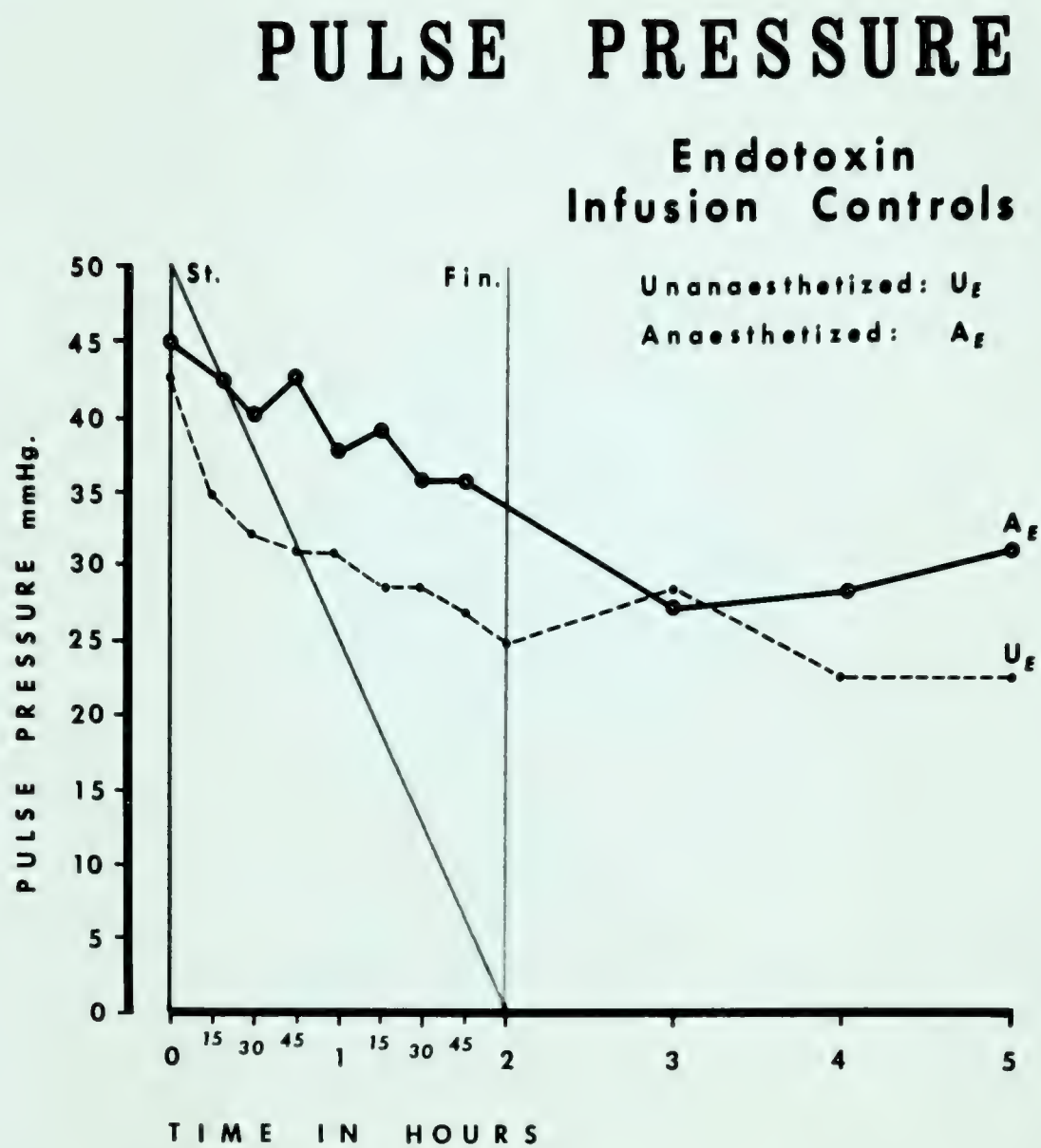


Figure 11.

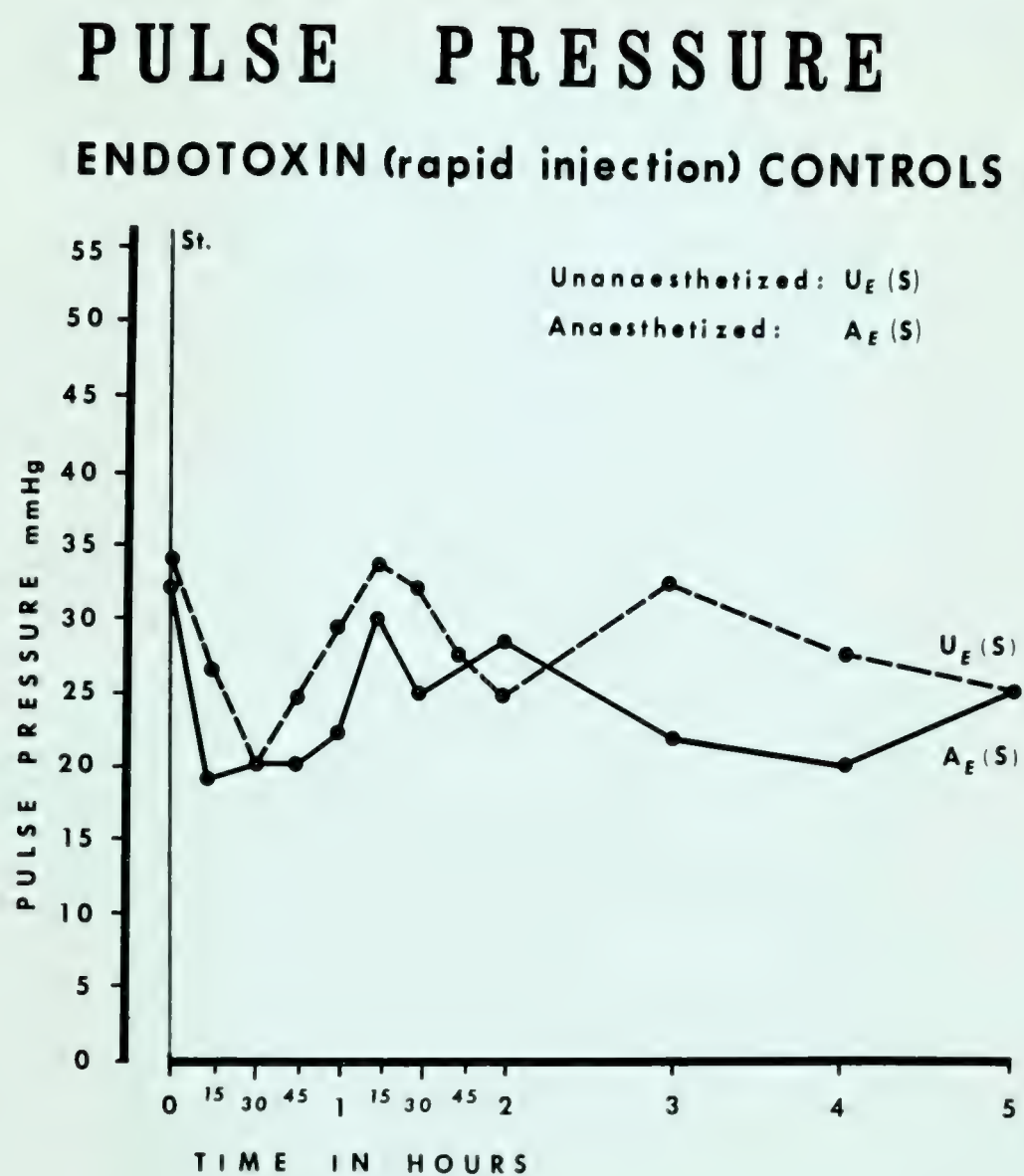


Figure 12.

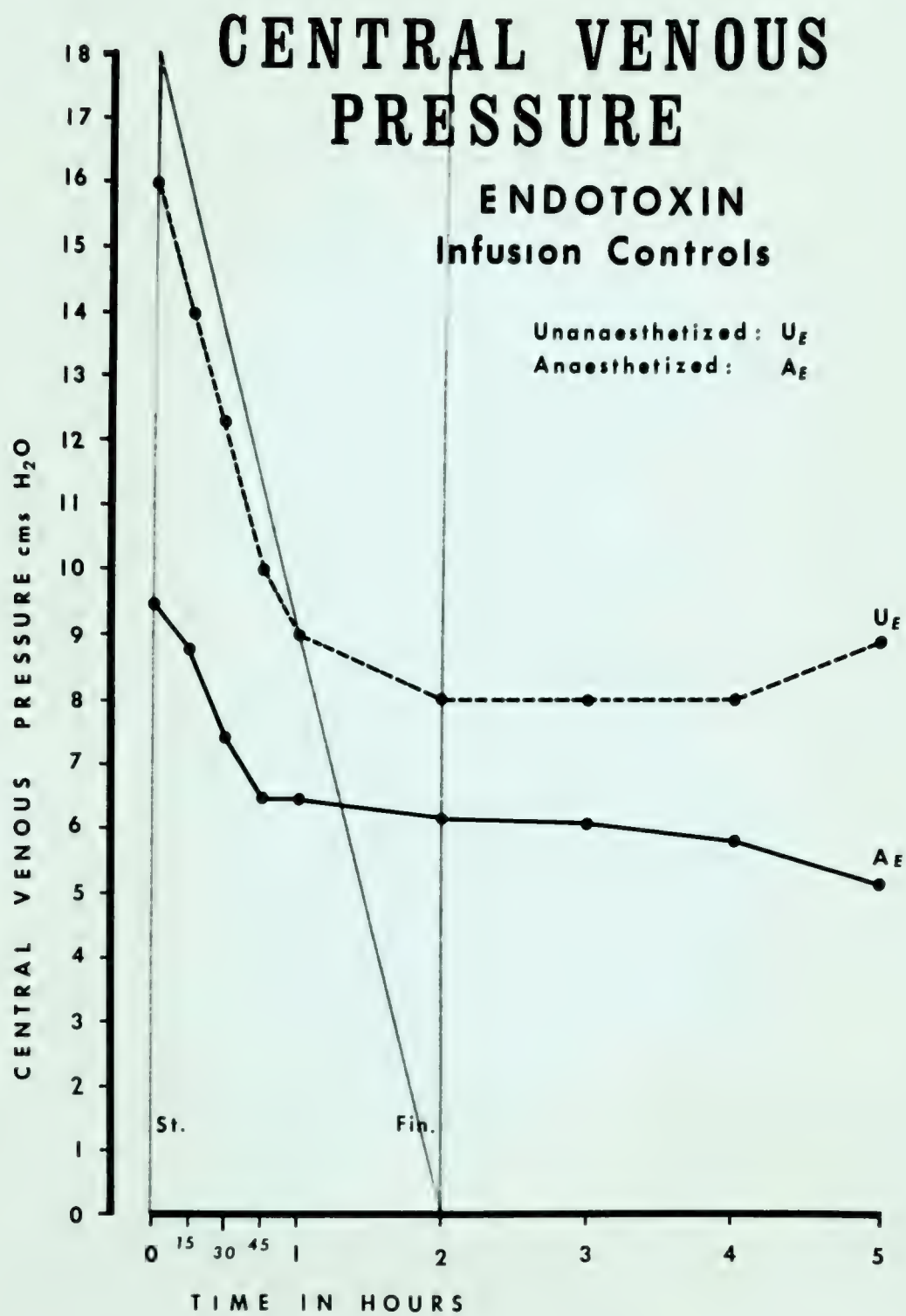


Figure 13.

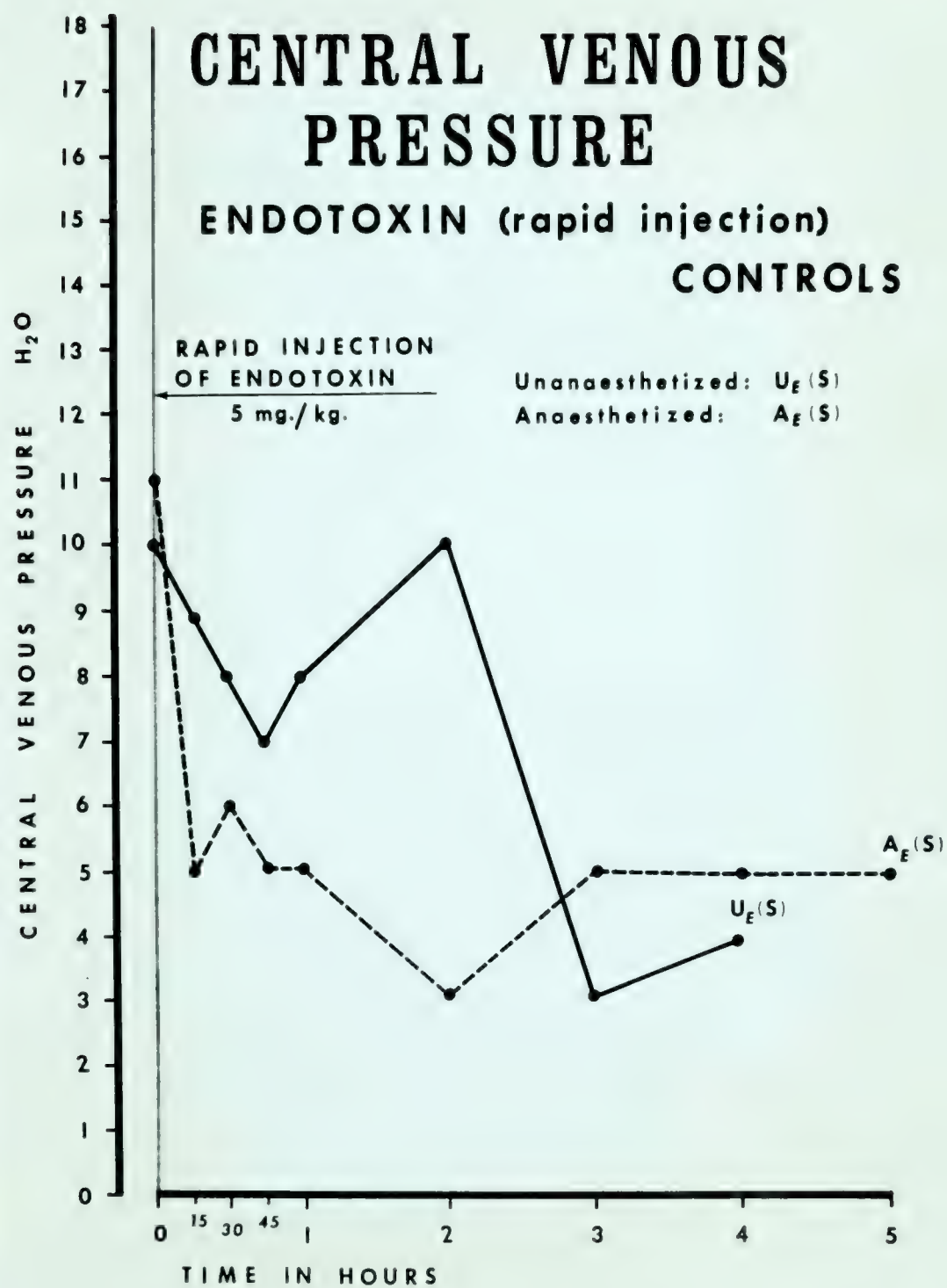


Figure 14.

HIND LIMB VENOUS OUTFLOW

ANAESTHETIZED CONTROLS -

- ENDOTOXIN INFUSION : A_E
- ENDOTOXIN RAPID INJECTION : $A_E(S)$
- T R E A T M E N T : A_T

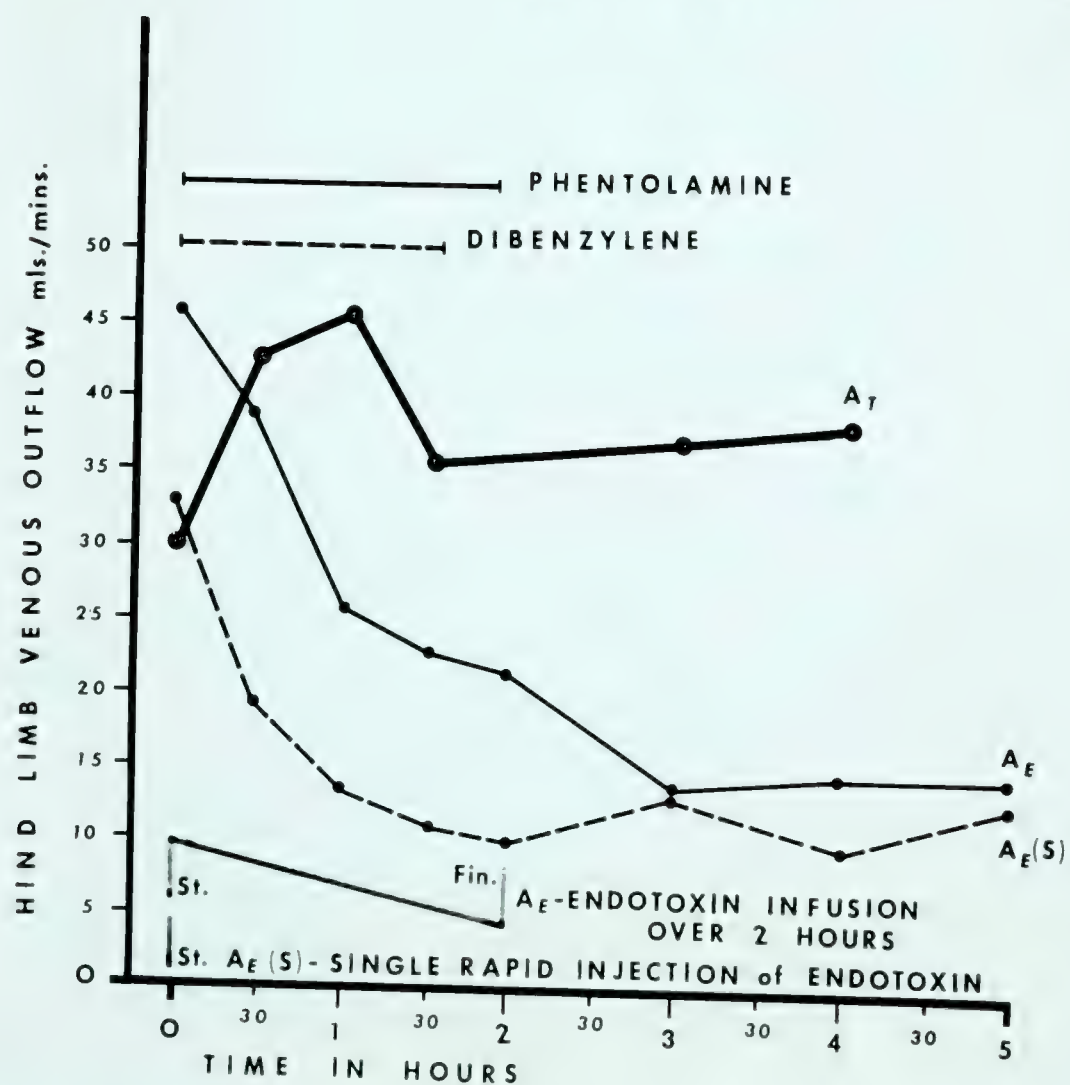
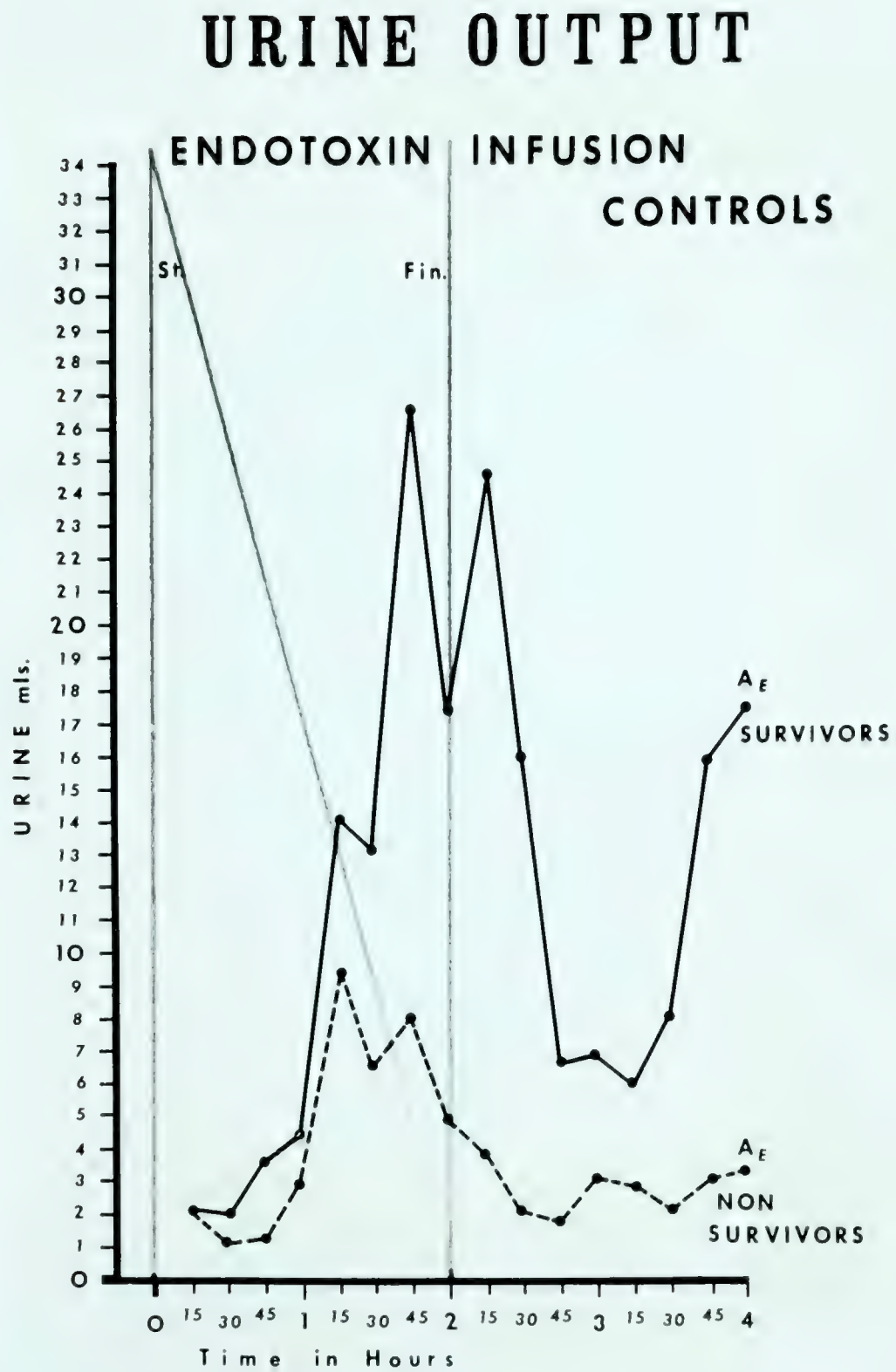


Figure 15.



the renal vessels increases at first, and decreases later. In his view, endotoxin probably does not affect renal function per se, except indirectly by way of the renal blood flow.

Another mechanism which may be responsible for the late diuresis in the course of endotoxin infusion is a fall in the secretion of antidiuretic hormone.

Another likely explanation is that the diuretic response is the direct result of the fluid volume in which the endotoxin was administered. This is an undesirable feature of the method of endotoxin infusion used in this study.

Coincidentally with this diuretic response in the second hour of endotoxin infusion the pH of the arterial blood is seen to rise temporarily after an initial fall. (Fig. 16). The excretion of acid radicals during diuresis may explain this fact.

The changes in arterial pH following slow infusion and rapid injection of endotoxin are illustrated in figures 16 and 17.

The changes in arterial $p\text{CO}_2$ are shown in figures 18 and 19, and those in arterial HCO_3 in figures 20 and 21.

The results quoted above illustrate the mode of action of endotoxin on the circulation and on the acid-base balance of the dog.

When endotoxin is administered to the dog, it produces a marked vasoconstrictor response, particularly on the hepatic

Figure 16.

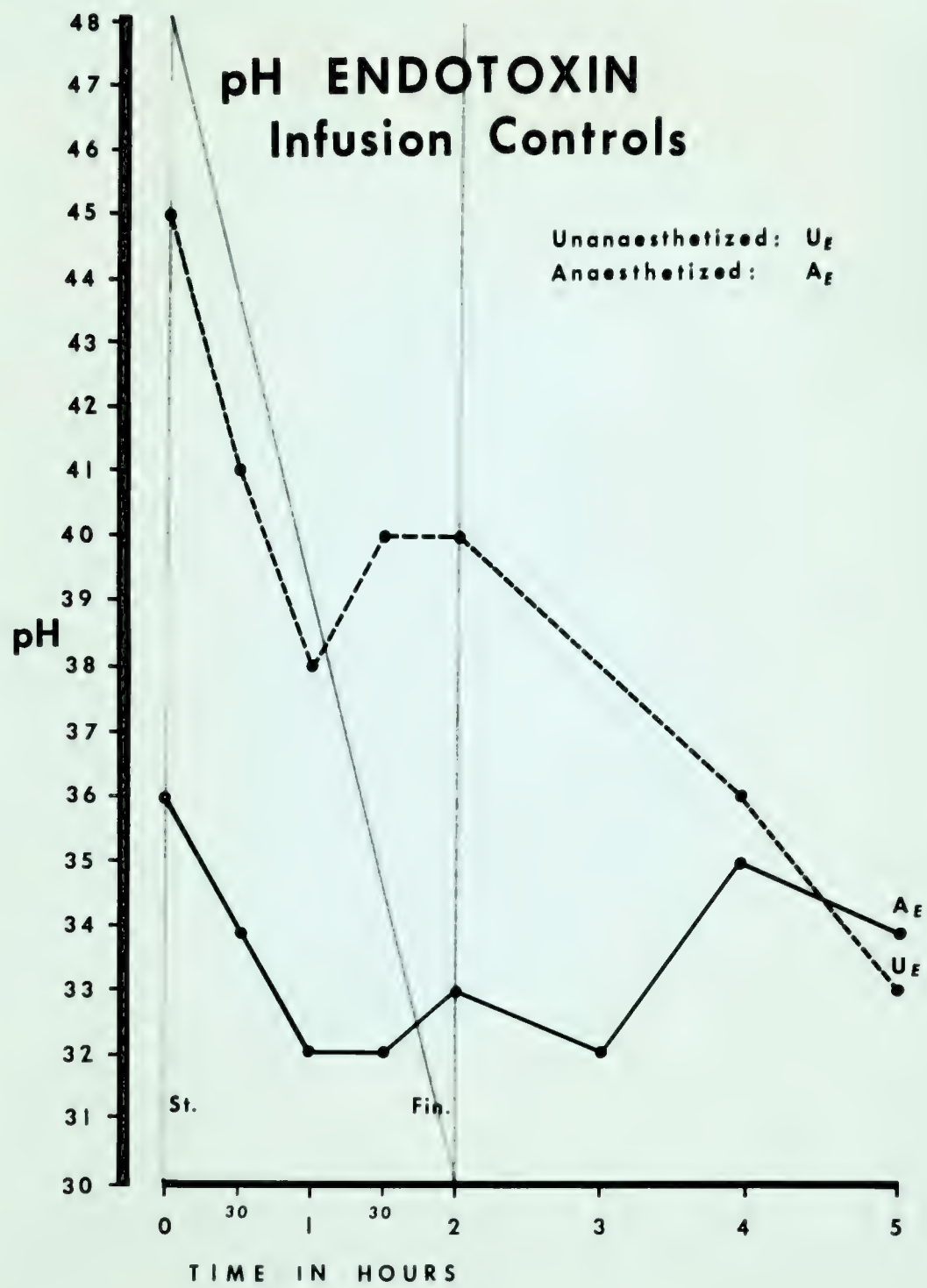


Figure 17.

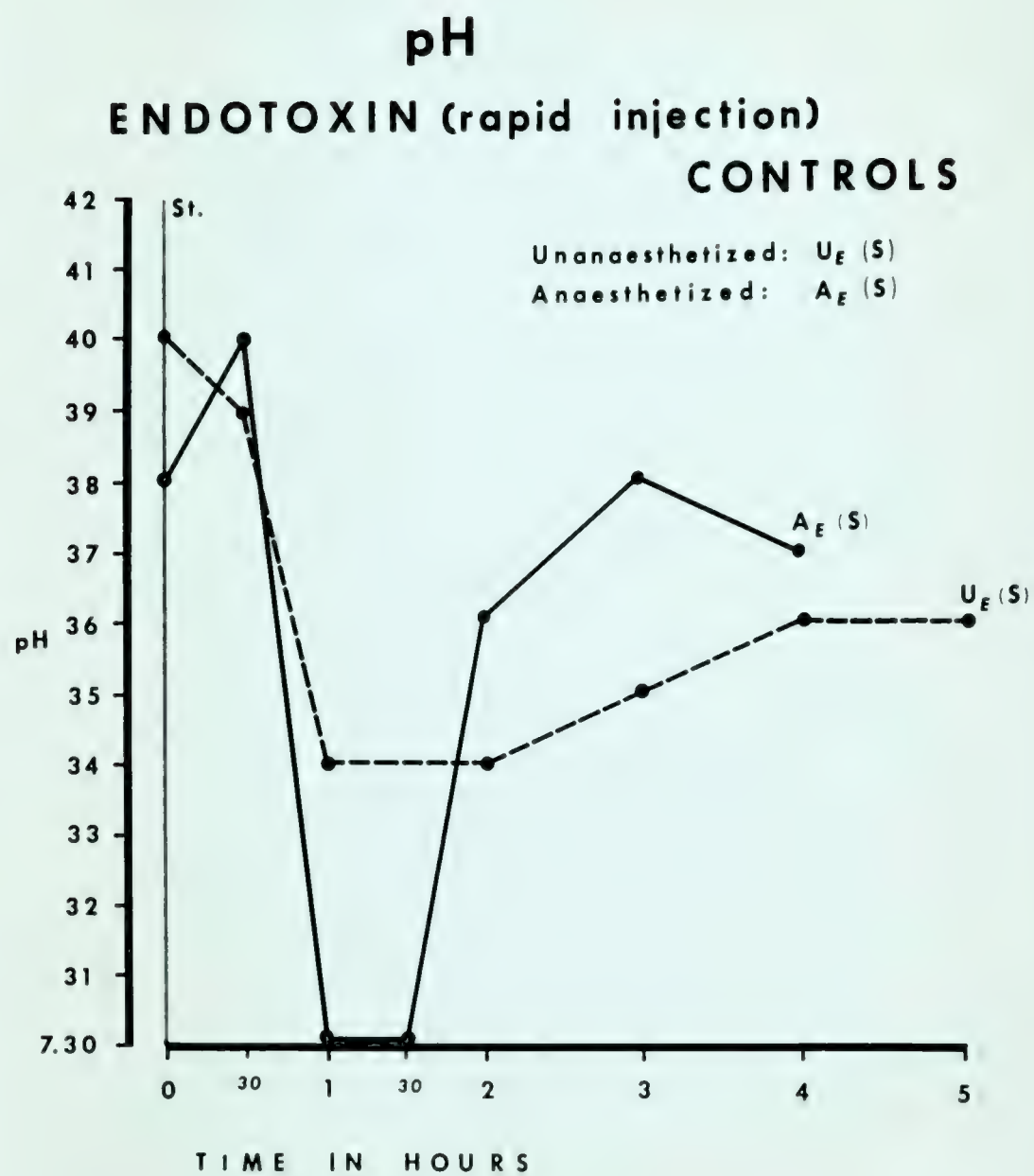


Figure 18.

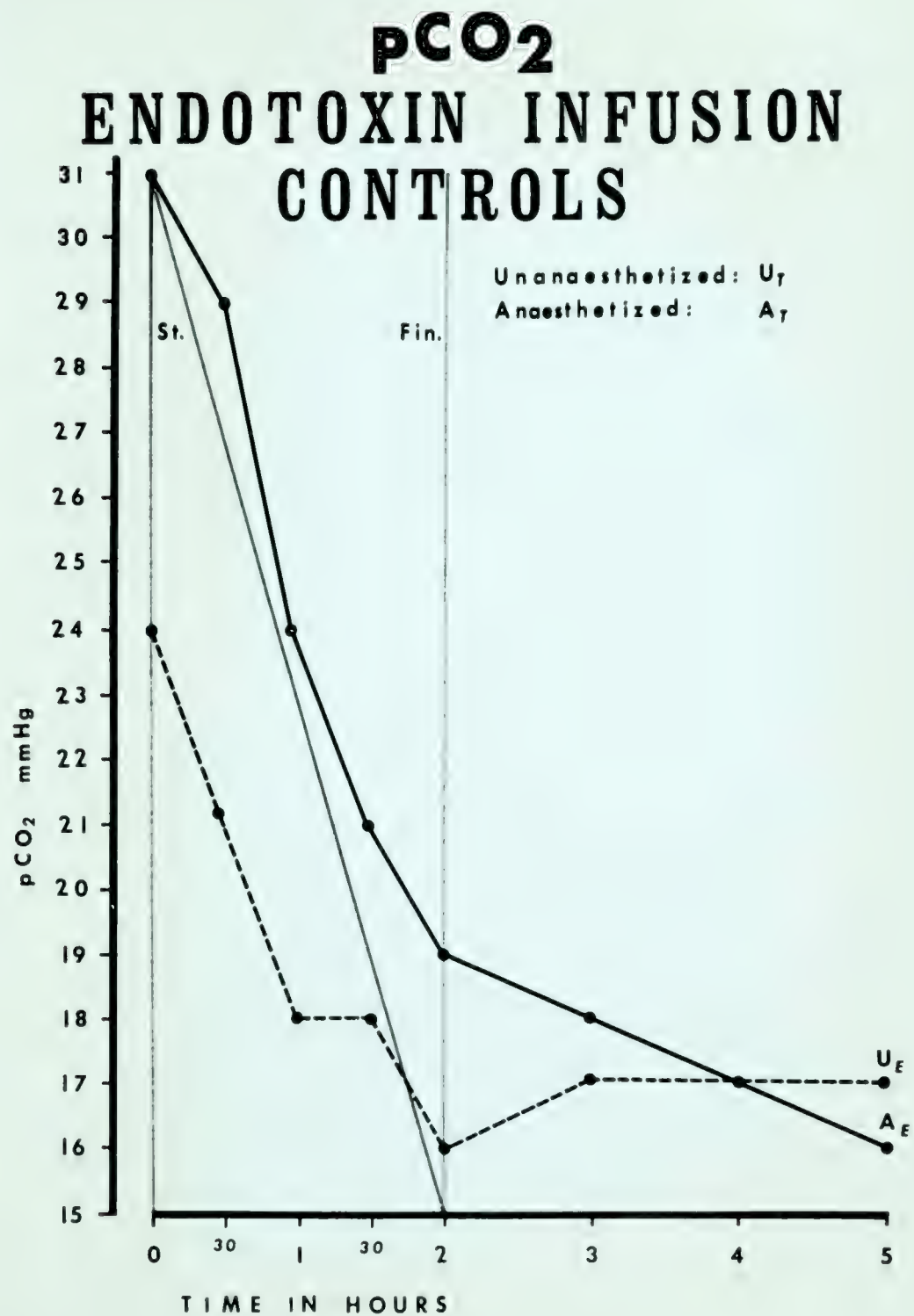


Figure 19.

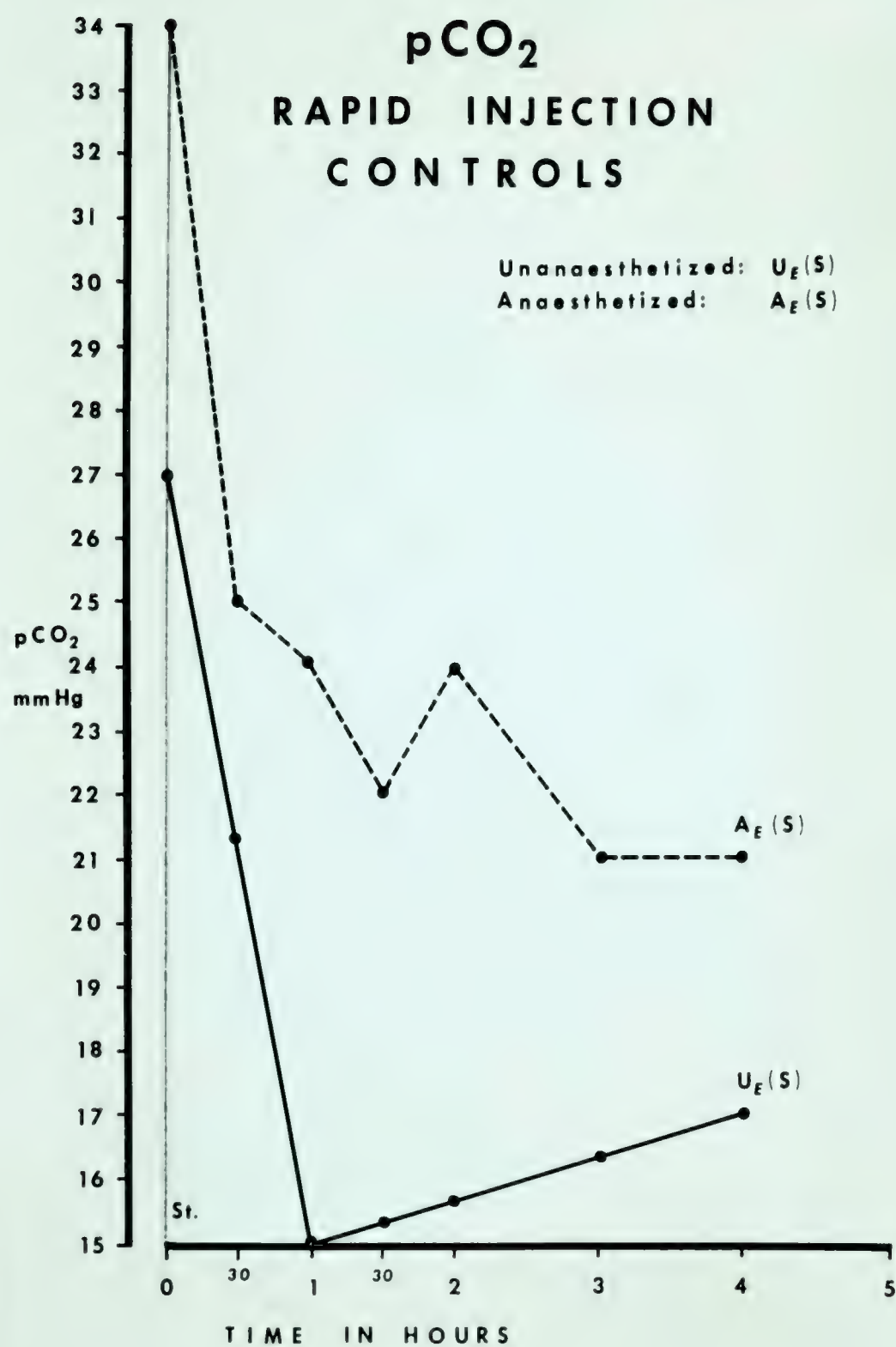


Figure 20.

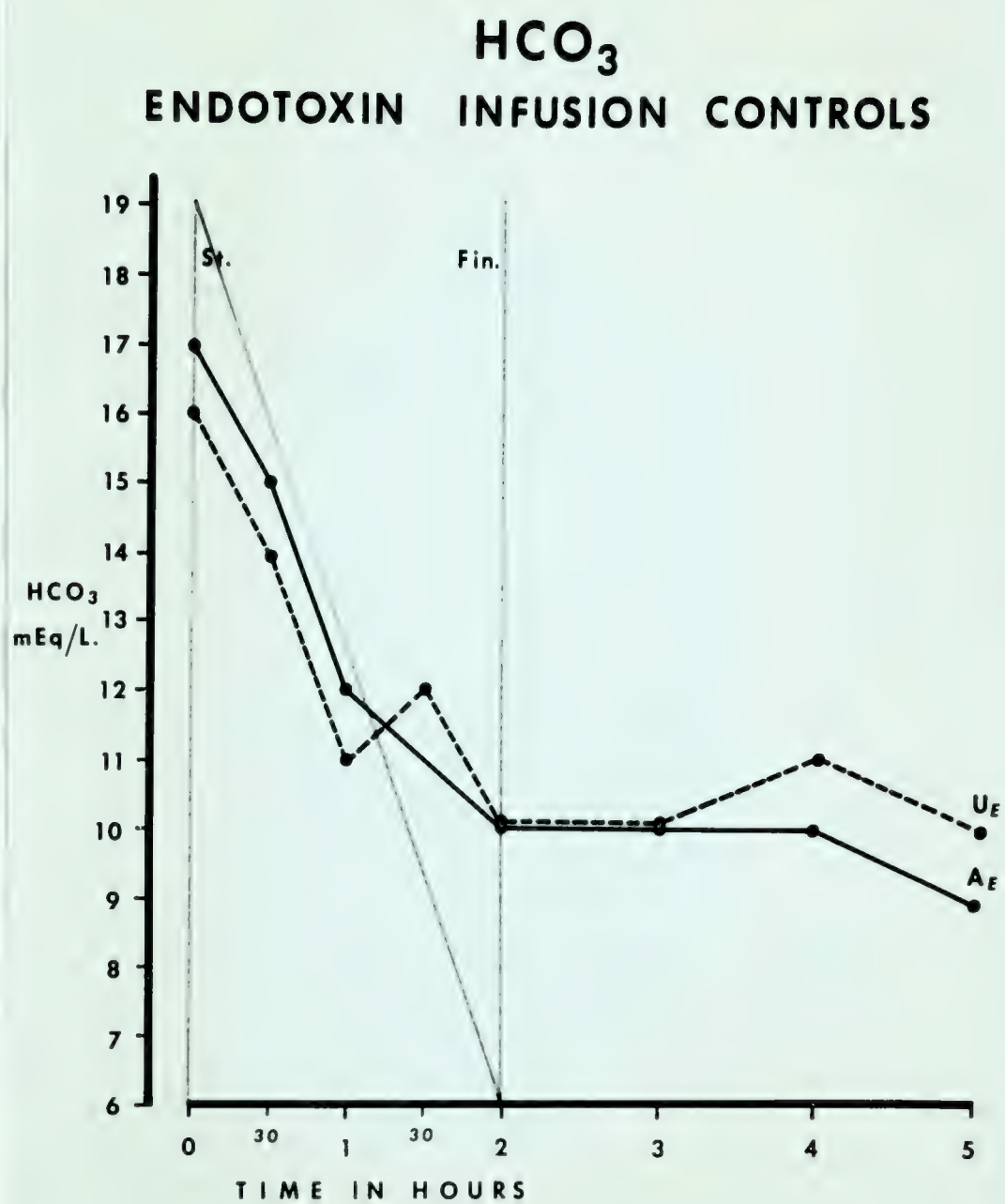
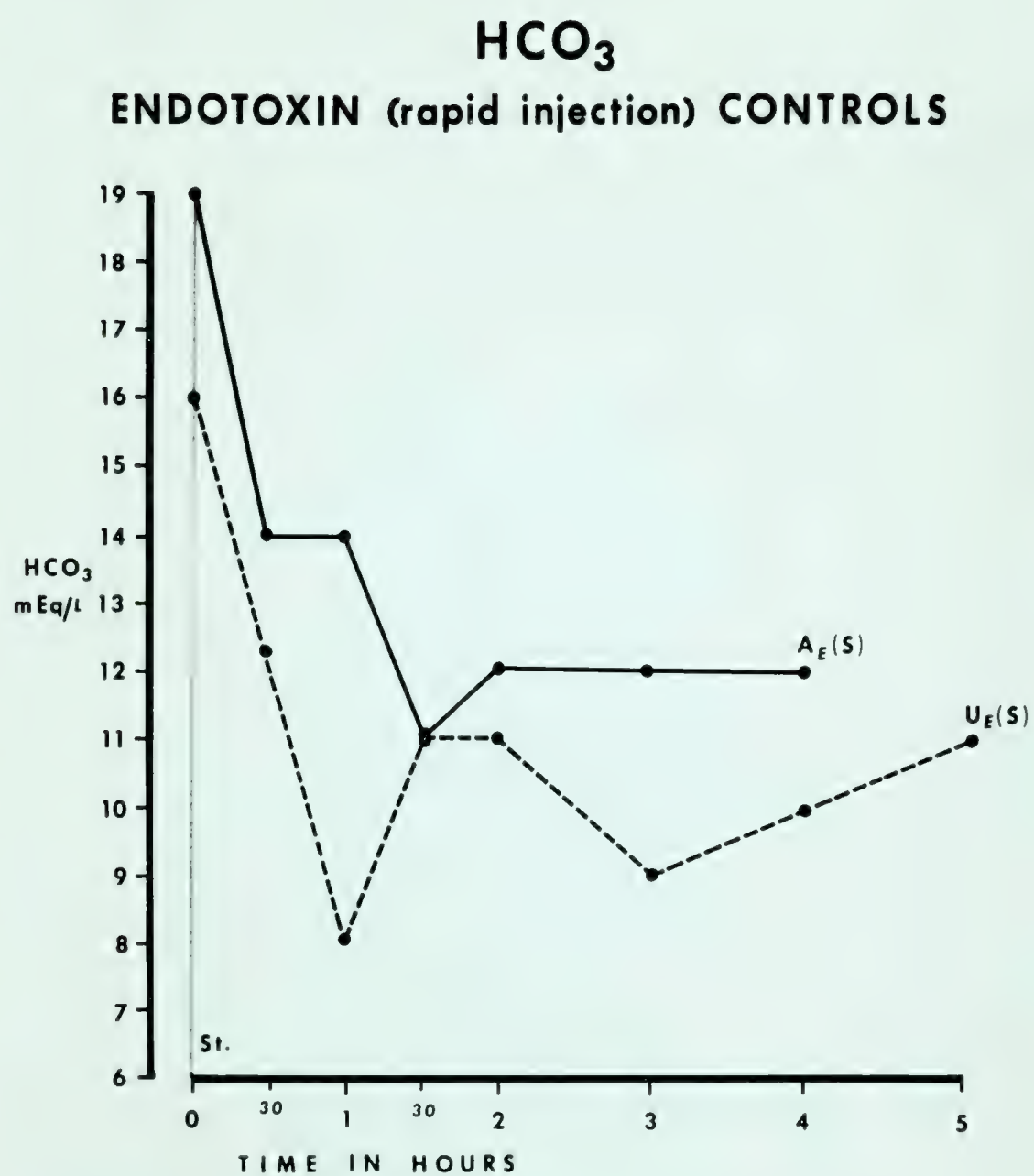


Figure 21.



veins and venules and on the intestinal circulation. As a result, the venous return is diminished. This results in a fall in central venous pressure. The restriction of venous outflow from the capillary network increases the filtration pressure in the capillaries with resultant loss of fluid from the intravascular compartment. This augments the deficit in venous return. The central venous pressure thus falls.

Because of the diminution in venous return the cardiac output is reduced. This probably explains the fall in mean blood pressure. Tachycardia sets in and the pulse pressure falls. The hind limb venous outflow is reduced both as a result of peripheral vasoconstriction and the fall in cardiac output.

The reduced perfusion of the tissues results in the formation of lactic acid in large quantities. This jeopardizes the pH of the arterial blood. Hyperventilation compensates for this hazard by the elimination of CO_2 . At the same time HCO_3 buffer is used up in an attempt to sustain the pH at normal levels.

The results obtained justify the method of producing experimental endotoxaemia by slow infusion of endotoxin over two hours. The deterioration in the parameters was steady, comparable in extent with, or even exceeding that produced by rapid injection. The changes seen with slow infusion, even before the full dose has been administered, show that even in small doses, endotoxin has a powerful effect on the circulation. It may be remembered that Zweifach showed that

prolonged infusion of a subthreshold dose of endotoxin in the isolated, perfused rabbit ear dramatically enhanced the sensitivity of the blood vessels to epinephrine (122).

The slow infusion is likely a better reproduction of the clinical picture. It demonstrates that the blood pressure of the dog during the development of endotoxaemia exhibits the same gradual decline as the blood pressure of man.

It may be said that the rapid injection of endotoxin can be compared to the throwing of the weights into the pan of a measuring scale. Eventually the balance will be found but not before initial fluctuations which only serve to confuse. In addition it may be argued that the sudden trauma of a massive injection produces unnecessary harm which is due more to the method of administration than to the endotoxin itself.

The method of infusion of endotoxin used in this study may be criticized on the grounds that fluid volume is being administered to the dog with the endotoxin. This criticism is justified. However, the 250 mls of fluid used to convey the endotoxin was considered a reasonable minimum volume which could be infused at an even rate over 2 hours, using the conventional intravenous giving sets.

The method of infusion used in this study can be improved considerably by suspending the dose of endotoxin in a volume of 20-25 ccs. of intravenous fluid and administering this volume to the dog in two hours by means of a Harvard pump.

This would serve to eliminate the infusion of extra fluid volume used as a vehicle for endotoxin, which may have alleviated the haemodynamic disturbances in the present study.

The unanaesthetized v. the anaesthetized dog.

This phase of the study sought an answer to the question whether there is an adverse effect of anaesthesia on the parameters measured as compared with the parameters studied in unanaesthetized dogs. This was an important question to settle, since the experiment on the anaesthetized animal is much more convenient and yields more information, while that on the unanaesthetized dog provides fewer parameters and is beset with frustrations.

The effect of endotoxin infusion on the mean blood pressure in the two groups is illustrated in Figure 8. The average reading in both groups was virtually identical at the start. The deterioration was equal in the first hour of the infusion. Thereafter the blood pressure in the anaesthetized group continued to fall, while it remained stable in the unanaesthetized dogs. At 3 hours the difference in the two groups was statistically significant at the $p = \text{less than } 0.05$ level. This would indicate that anaesthesia at that time in the experiment enhanced the sympathomimetic effect of endotoxin in the anaesthetized group. In the subsequent 2 hours, however, some degree of compensation was achieved by this group and the

blood pressure rose to a level approximating that in the un-anaesthetized animals. At 5 hours there was no statistically significant difference in the mean blood pressure level between the two groups.

There was no significant difference between the two groups in pulse pressure (Fig. 10) as a result of endotoxin infusion.

The central venous pressure (Fig. 12) exhibited a more profound fall in the unanaesthetized group during the first hour of endotoxin infusion. This may have been due to the hyperpnoea which was typical of this group during this period. After the first hour both groups showed parallel readings.

The anaesthetized group exhibited a higher arterial pCO_2 level and a lower arterial pH at the start of the experiment. This is probably due to the hypoventilation in these dogs during anaesthesia. At the end of 5 hours pCO_2 and pH levels in the two groups were virtually identical. (Figs 16 and 18).

The HCO_3 levels in the two groups remained closely parallel in both anaesthetized and unanaesthetized dogs during the five hours of measurement. (Fig. 20).

Comparison of the parameters in the unanaesthetized and anaesthetized endotoxin control groups which received endotoxin by rapid injection shows close similarity to the above findings. The more profound initial drop in the mean blood pressure in the unanaesthetized group is possibly an accentuation of hypotension due to dependence of the limbs.

In this context the recent work of Weil and his group

(111) is worthy of mention. Working with rats and mice they showed that in shock due to *E. coli* endotoxin the head-down position adversely affected survival. They reason that the disadvantages of the head-down position are a delayed return of postural reflexes, reduction of effective lung volume by pressure of the viscera against the diaphragm, and the risk of cerebral oedema. In shock due to endotoxin, where pooling of blood occurs in the splanchnic venous bed, distal to constricted splanchnic venules and veins, it is not likely that the assumption of the head-down position will increase vena caval return flow.

Review of the results quoted above would seem to indicate that there is no significant difference in the parameters studied between unanaesthetized and anaesthetized dogs.

The difficulties and undesirable features of the "unanaesthetized" experiment were outlined earlier.

The introduction of anaesthesia of short duration for the preparation of the dog on the day of the "unanaesthetized" experiment was a great improvement. The dogs appeared fully recovered from the effects of thiopentone after 60-90 minutes and were stable when the experiment began. No cannula was dislodged by accident and no experiment was forfeited. The urinary catheter - most difficult to protect during the overnight preparation - was safe.

However, even with the improved method of preparation for the "unanaesthetized" experiment, it is still inferior to

the experiment conducted under anaesthesia. The "anaesthetized" experiment is much easier to perform. Under anaesthesia the dogs are stable, and not influenced by the environment. In the anaesthetized dog a larger number of parameters can be measured and it may be assumed that all the changes observed are due to the measured insult to which the animal is subjected. Moreover, there is no wastage of dogs through accidental hazards.

Another advantage of the "anaesthetized" experiment is that at its conclusion the cannulae may be removed and the vessels tied. The dog returns to the Vivarium with clean, closed surgical wounds. At the conclusion of the "unanaesthetized" experiment the best that can be done is to tie a knot in the polyethylene cannula close to the skin and cut the tubing just distal to the knot. Such an arrangement invites the spread of infection into the depth of the wound. Moreover, the dog may succeed in pulling out the cannula, with resultant bleeding. All these factors may adversely affect survival and will prejudice the true effect of endotoxin on this parameter.

Consideration of the facts discussed above leads to the conclusion that there is no advantage in the study of endotoxin shock in the unanaesthetized dog. Anaesthesia affords a more elegant experiment with more information and should be used in preference.

Treatment controls.

Four dogs in both the anaesthetized and unanaesthetized groups were given treatment (i.e. phentolamine and dibenzylene) alone. All of these dogs survived.

The pulse pressure in the anaesthetized group showed a marked, but transient widening in the first 2 hours. (Fig. 22). This was found to be due to lowering of the diastolic pressure in these dogs. This may be an indication of the success of adrenergic blockade. After 2 hours the pulse pressure fell to below pre-experiment levels. The pulse pressure in the unanaesthetized group was hardly affected at all. At 1 hour the difference between the two groups is statistically significant at the $p = \text{less than } 0.05$ level. The difference demonstrated between the two groups may be due to the ability of the unanaesthetized dog to compensate for the disturbance in vasoconstrictor tone caused by the adrenergic blocking agents.

The hind limb venous outflow in the anaesthetized treatment control dogs showed a marked increase in the first hour, after which, though it exhibited a fall, it stayed at above pre-experiment levels during the next three hours. (Fig. 14). The cardiac stimulant action of phentolamine, in association with the increased flow which one would expect from adrenergic blockade, probably accounts for this change.

The mean blood pressure (Fig. 23), central venous pressure (Fig. 24), arterial pH (Fig. 25), arterial $p\text{CO}_2$ (Fig. 26) and arterial HCO_3 (Fig. 27) showed no significant change as a

Figure 22.

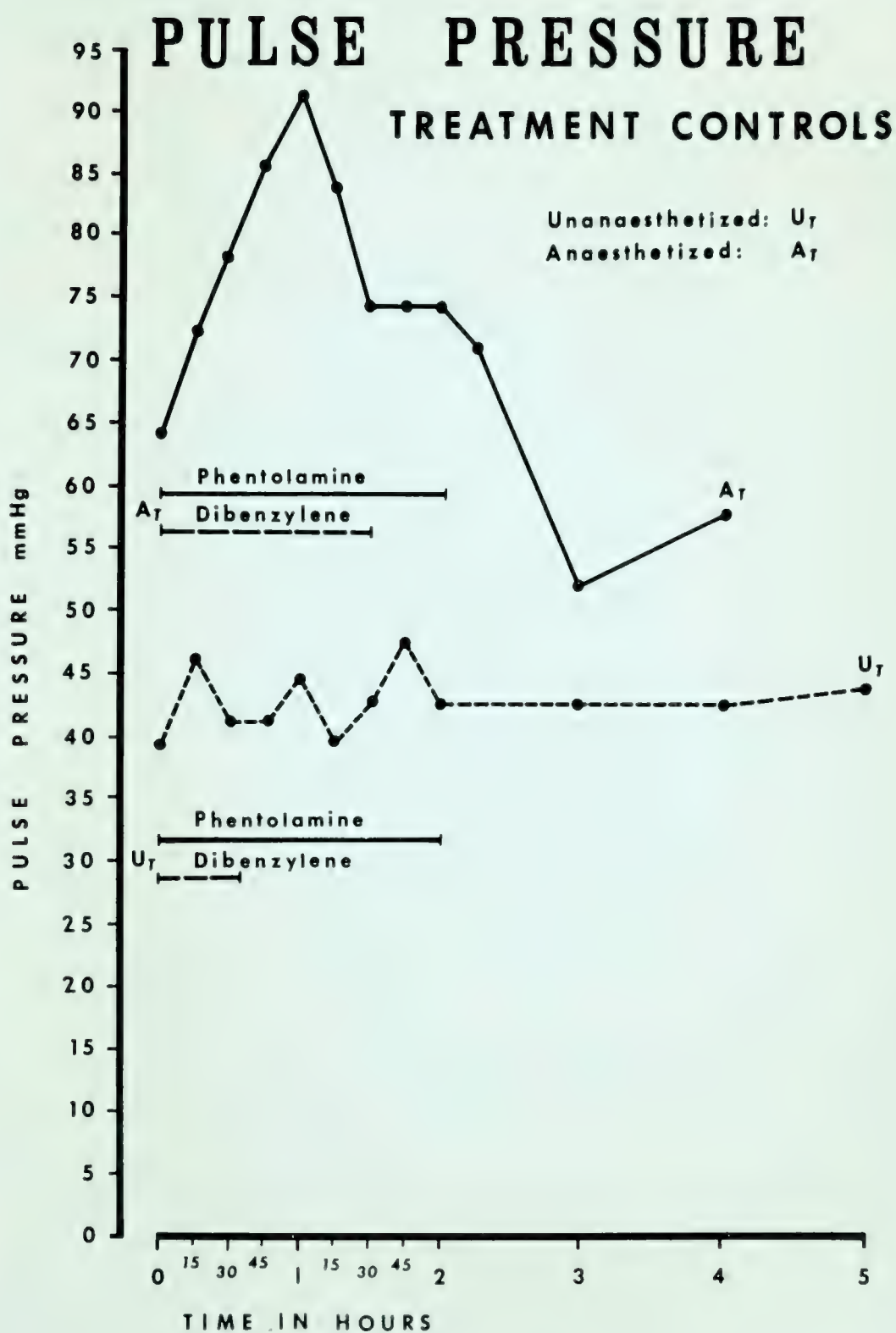


Figure 23.

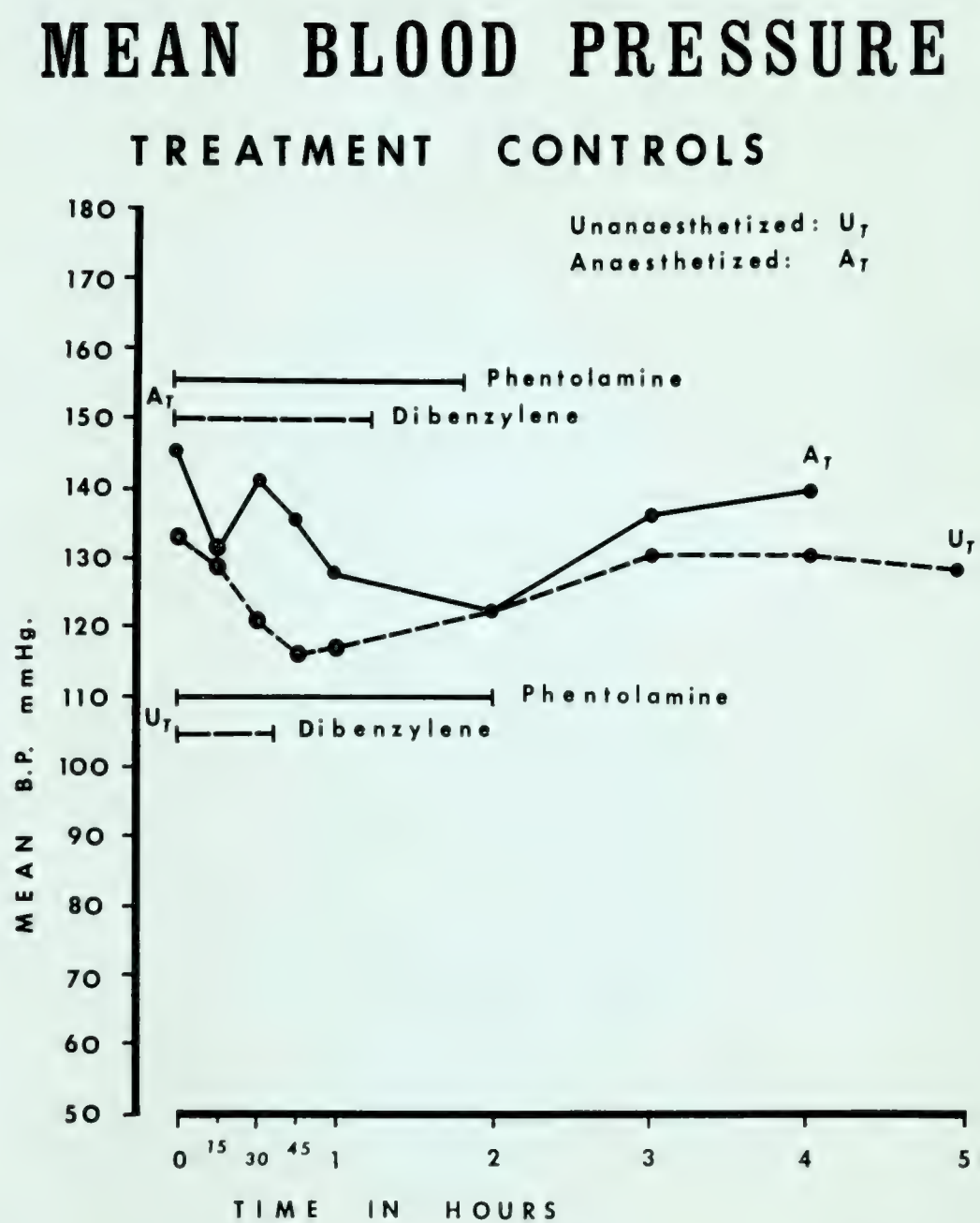


Figure 24.

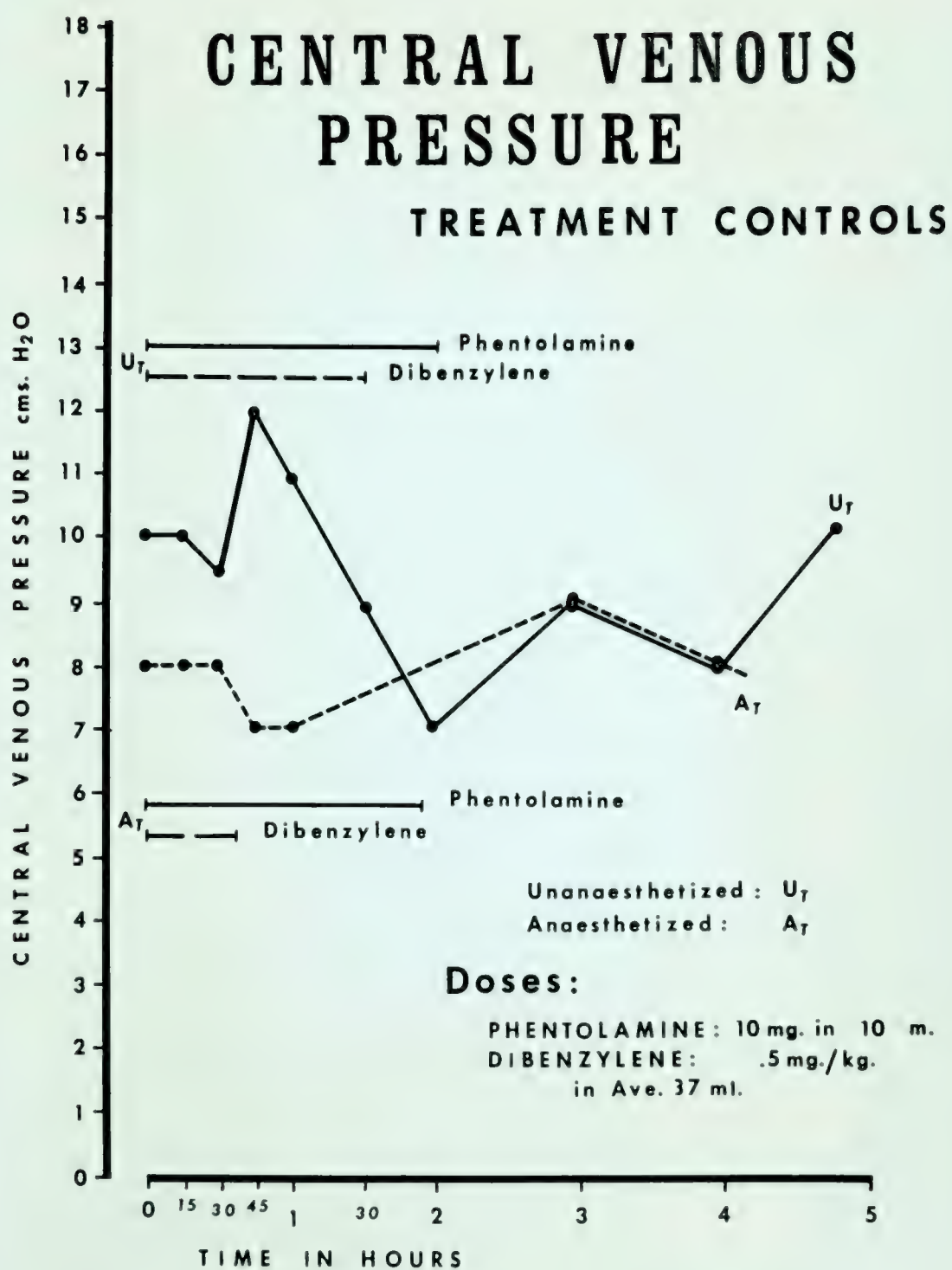


Figure 25.

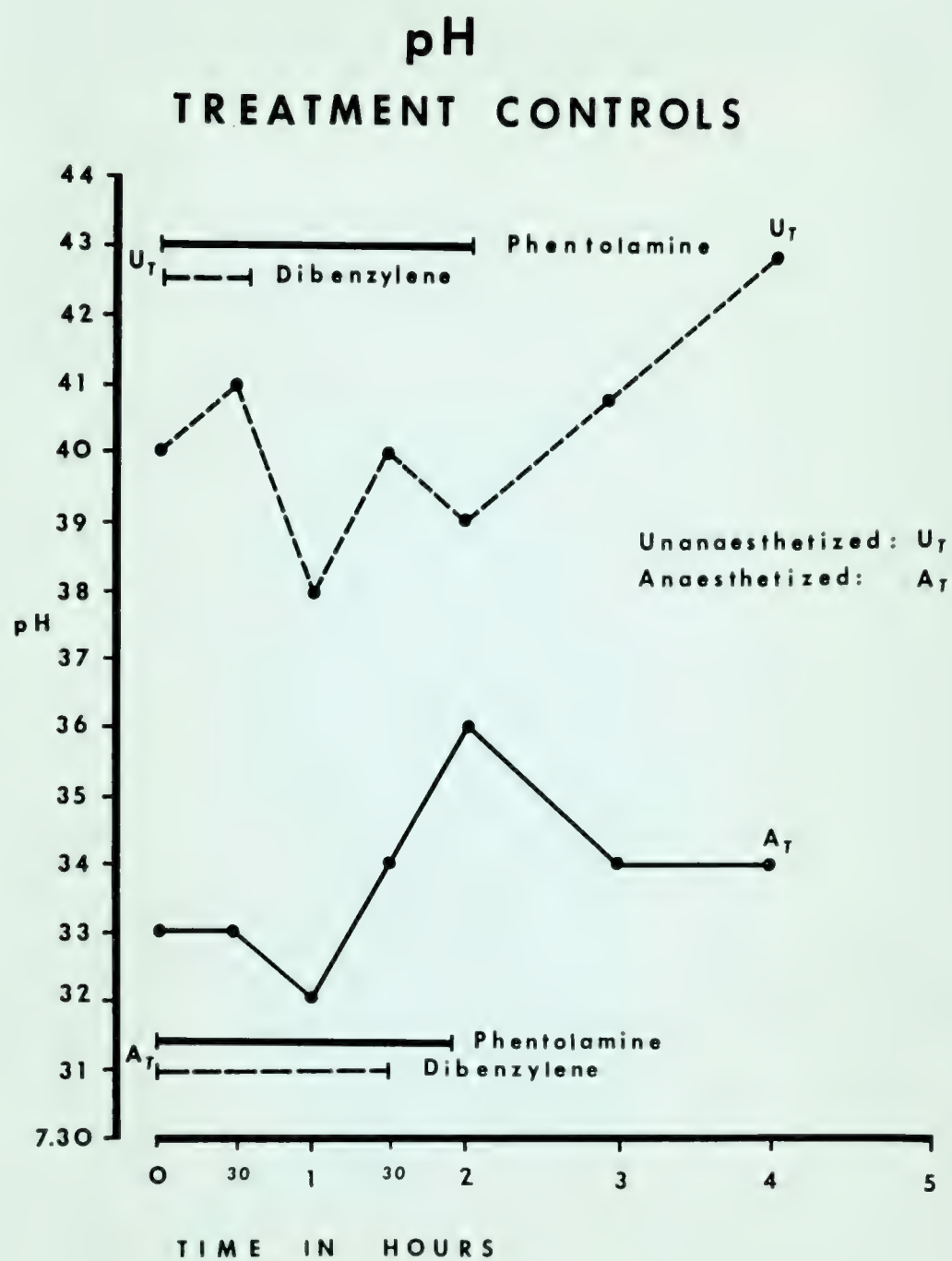


Figure 26.

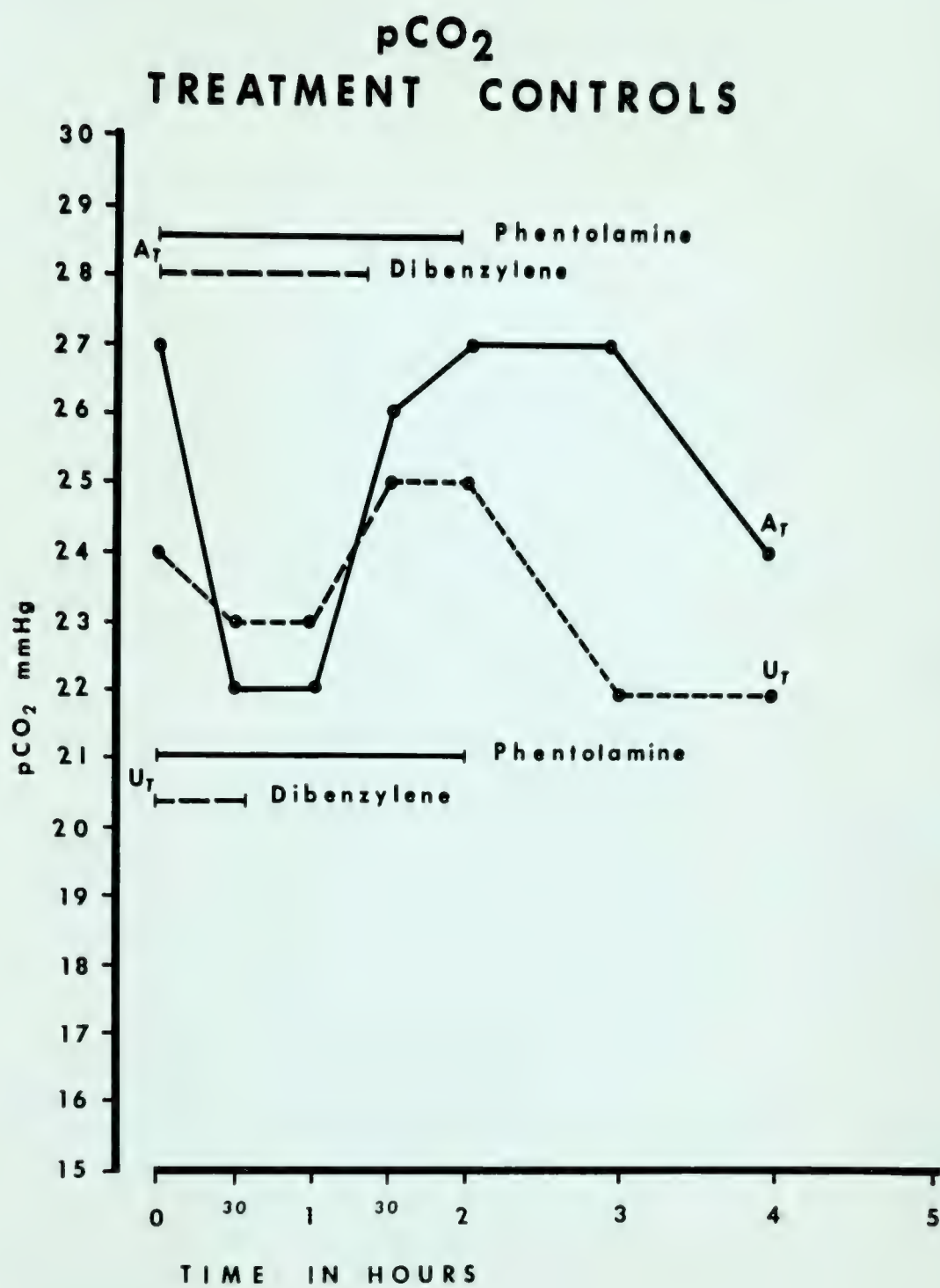
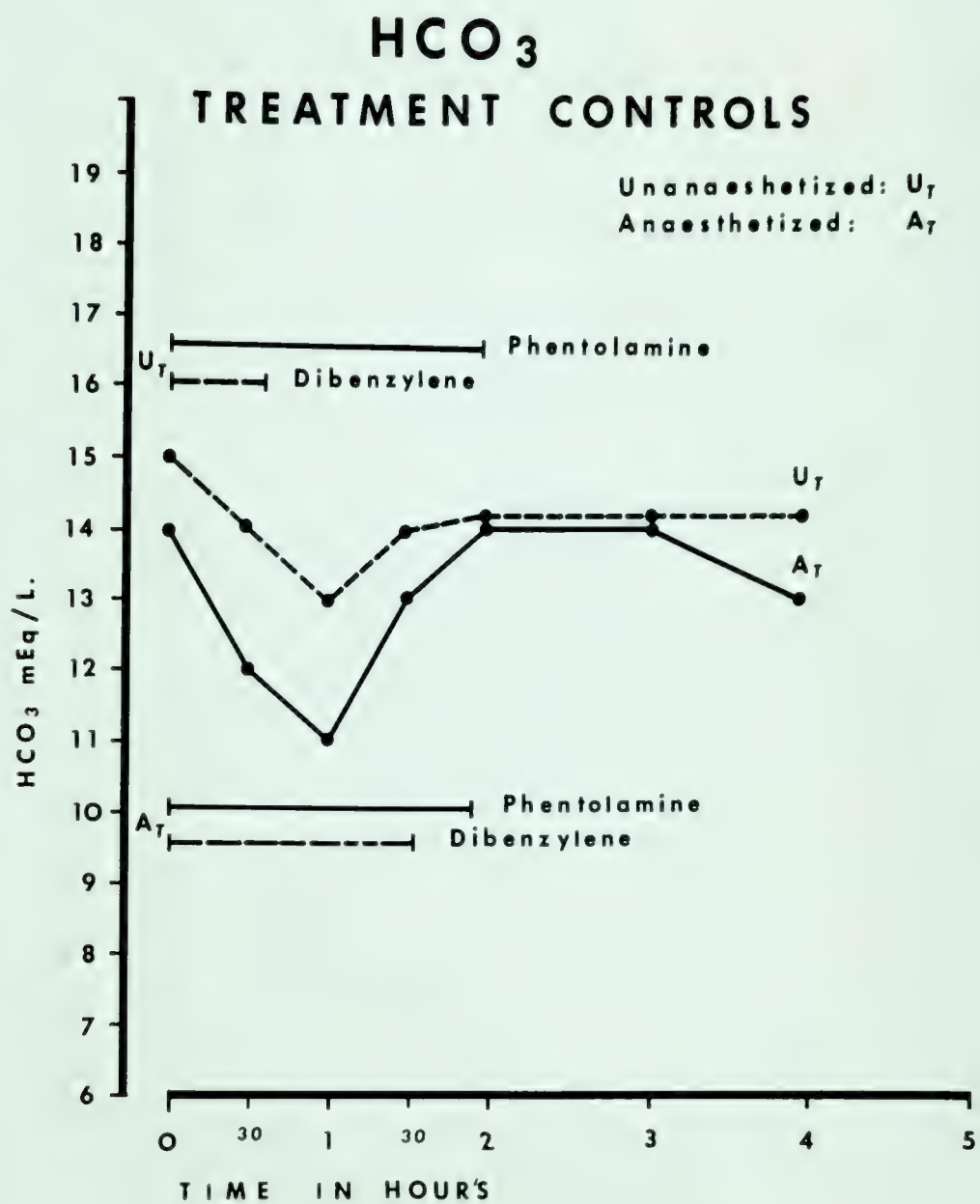


Figure 27.



result of adrenergic blockade in the normal dog.

Changes in parameters with treatment after endotoxin.

Treatment consisted of the simultaneous infusion of phentolamine and phenoxybenzamine. The former was infused over an average 90 minutes and the latter over a period of 45 minutes.

In the endotoxin control group (A_E) 5 dogs survived out of 15 while in the treated group (A_{ET}) 9 dogs out of 15 survived.

The use of adrenergic blocking agents during the phase of compensatory vasoconstriction in shock has a logical basis, as is evident from the discussion in Chapter I. In addition to facts presented in the earlier discussion, the concept of "critical closing pressure" merits consideration here. The critical closing pressure is that level of intraluminal pressure at which abrupt closure of the vessel lumen occurs. Its magnitude is determined by the amount of active tension of smooth muscle in the vessel wall, relative to the pressure within the lumen. Critical closing pressures rise to above normal values in cases of high vasomotor tone. Thus, in severe shock, when the intraluminal pressure is low, the active tension in the blood vessel wall may be sufficient to effect closure of the lumen. In regions where vasomotor tone is especially dominant large areas of circulation may be cut off in this manner. Tissue ischaemia and acidosis complicate this development. Once the vessels are closed, to

open them again pressures well in excess of the critical closing pressure are required. The reason for this is that with the vessel closed the mechanical advantage of the tension in the wall is at its maximum and it has to be overcome before the critical opening pressure is reached.

Exactly when during the development of severe shock critical closure of vessels occurs is not clear. There can be no doubt, however, that among the "many dry river beds" in the microcirculation, of which Fine spoke (19), many were arterioles that had undergone "critical closure".

Protection of the terminal vascular bed from excessive vasoconstriction in shock thus seems an urgent task. Dibenzylene is known to produce lasting adrenergic blockade, but a delay is incurred before its action is fully developed. This delay is added to the inevitable delay occasioned by the time taken for the perception of symptoms and signs and institution of treatment. If vasoconstriction could be abolished rapidly as soon as the condition is recognized, significant volumes of blood destined for pooling in the viscera may be salvaged. "Critical closure" of the arterioles would be prevented, thus maintaining the *vis a tergo* necessary for movement of blood in the capillaries and veins. At the same time it would safeguard the capillary bed from the stranglehold of capacitance vessel tone on the exit channels.

With the amelioration of the derangement in blood vessel tone the ischaemia and acidosis could be reversed.

The mean blood pressure in treated dogs is represented in Figure 28. At 3 hours the difference in pressure between survivors and non-survivors is statistically significant at the $p = \text{less than } 0.05$ level.

The pulse pressure showed a temporary increase in both groups. (Fig. 29). The lowering of diastolic pressure with adrenergic blockade is the likely explanation of this fact.

The hind limb venous outflow rose temporarily in both survivors and non-survivors in the first hour. (Fig. 30). Thereafter, in the non-survivors it fell to low levels.

The central venous pressure was not influenced by treatment. (Fig. 31).

The arterial pH showed marked divergence in survivors and non-survivors. (Fig. 32). In the former it showed a rise, while in the latter it exhibited a precipitous fall. At 3 hours the difference between the two groups is significant at the $p = \text{less than } 0.01$ level.

It is possible, however, that the demise of the non-survivors was accelerated by improvement of flow as a result of adrenergic blockade. If, with the opening up of the microcirculation, large volumes of stagnant blood, with high lactic acid content, are liberated, the pH of the blood will fall. At this stage the buffer reserves are low and may not be sufficient to correct the sudden acidosis. The arrival of acid radicals on the arterial side of the circulation will produce vasodilation in the arterioles. Vis a tergo is lost

Figure 28.

MEAN BLOOD PRESSURE in Treated Dogs

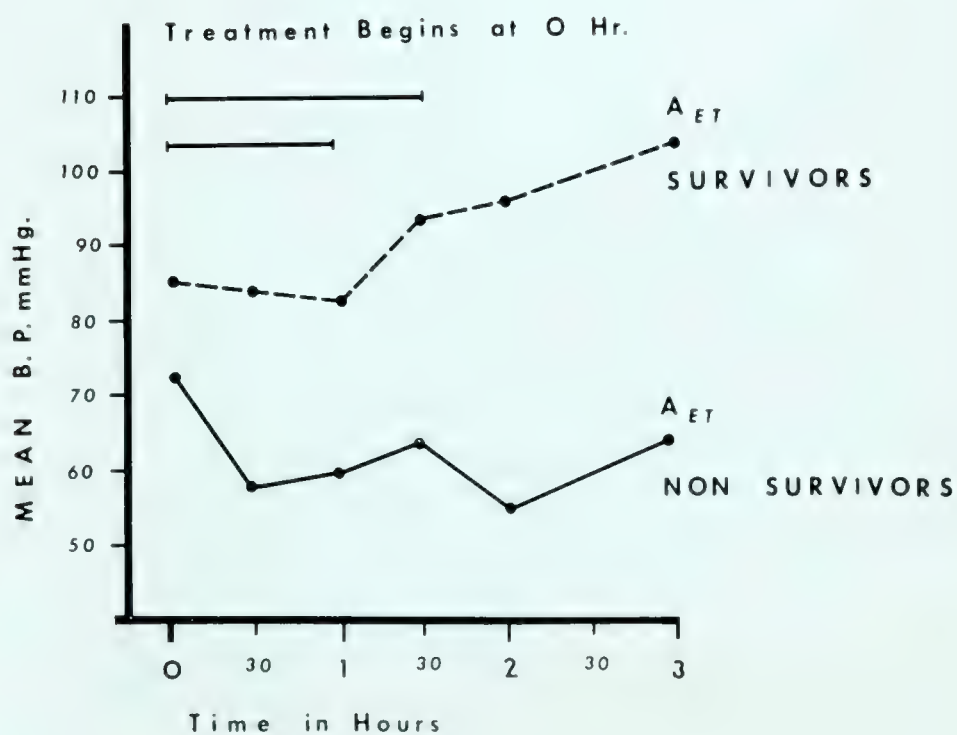


Figure 29.

PULSE PRESSURE in Treated Dogs

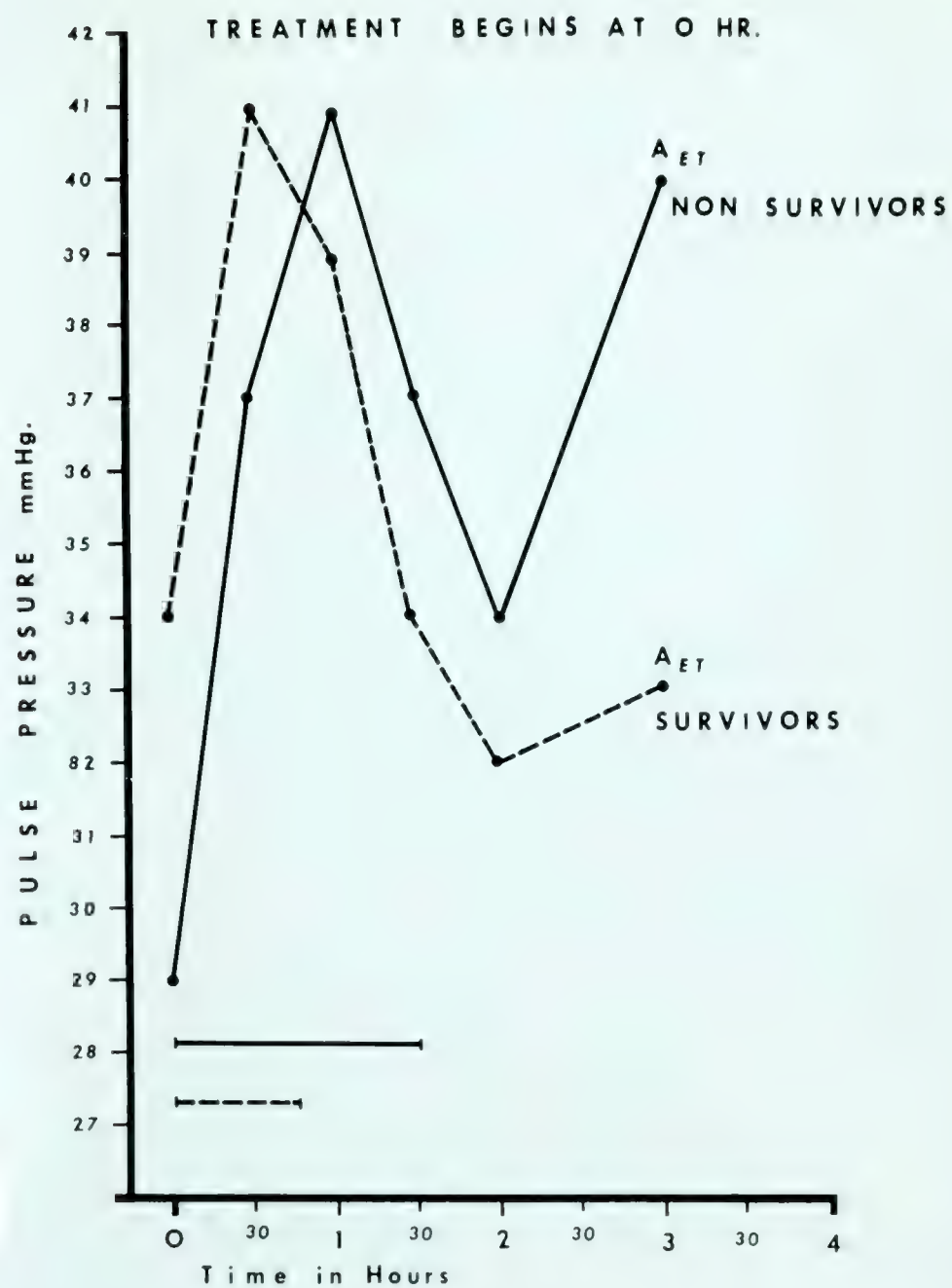


Figure 30.

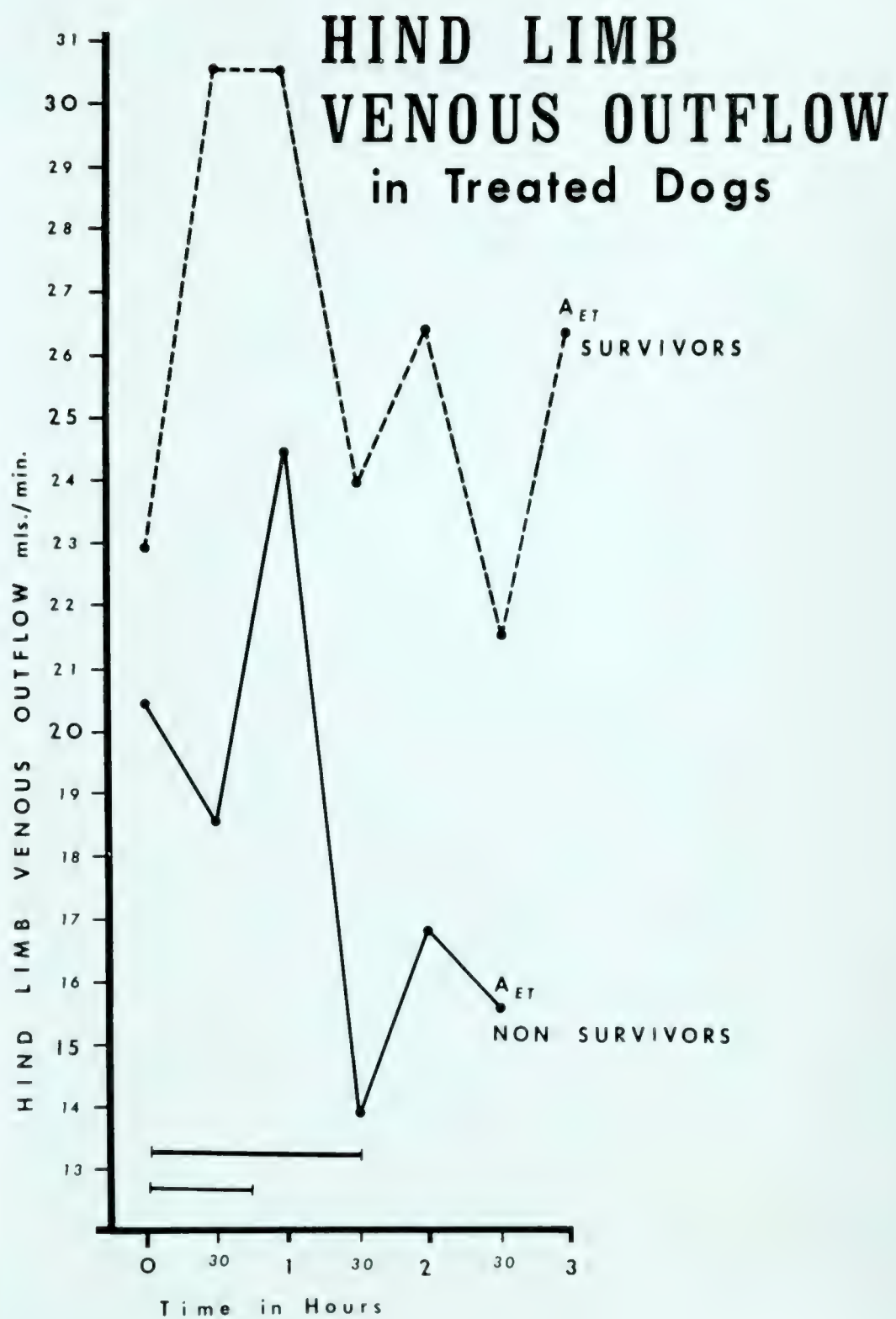


Figure 31.

CENTRAL VENOUS PRESSURE in Treated Dogs

TREATMENT BEGINS AT 0 HOUR

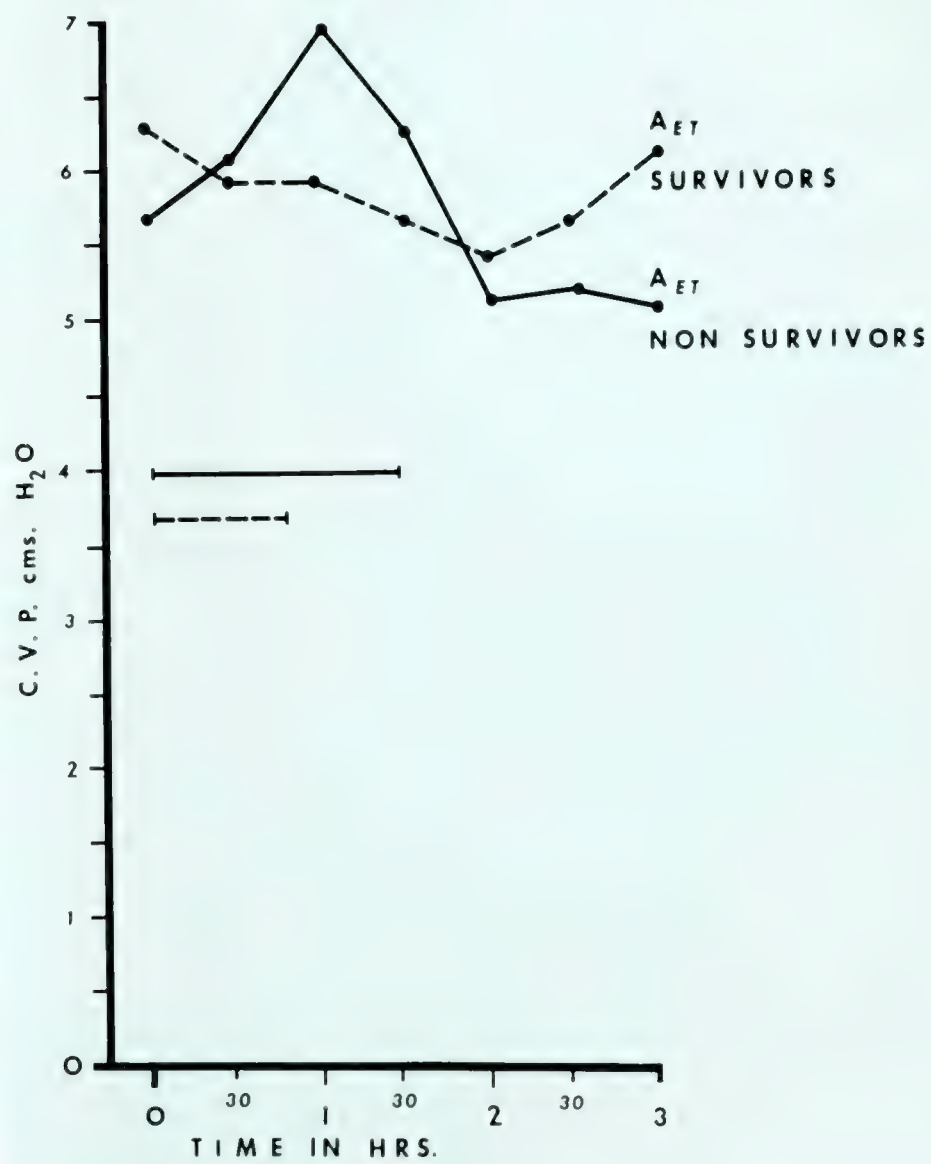


Figure 32.



and stagnation again occurs. This results in more acidosis and the dog succumbs.

The arterial HCO_3 levels in survivors and non-survivors are illustrated in Figure 33.

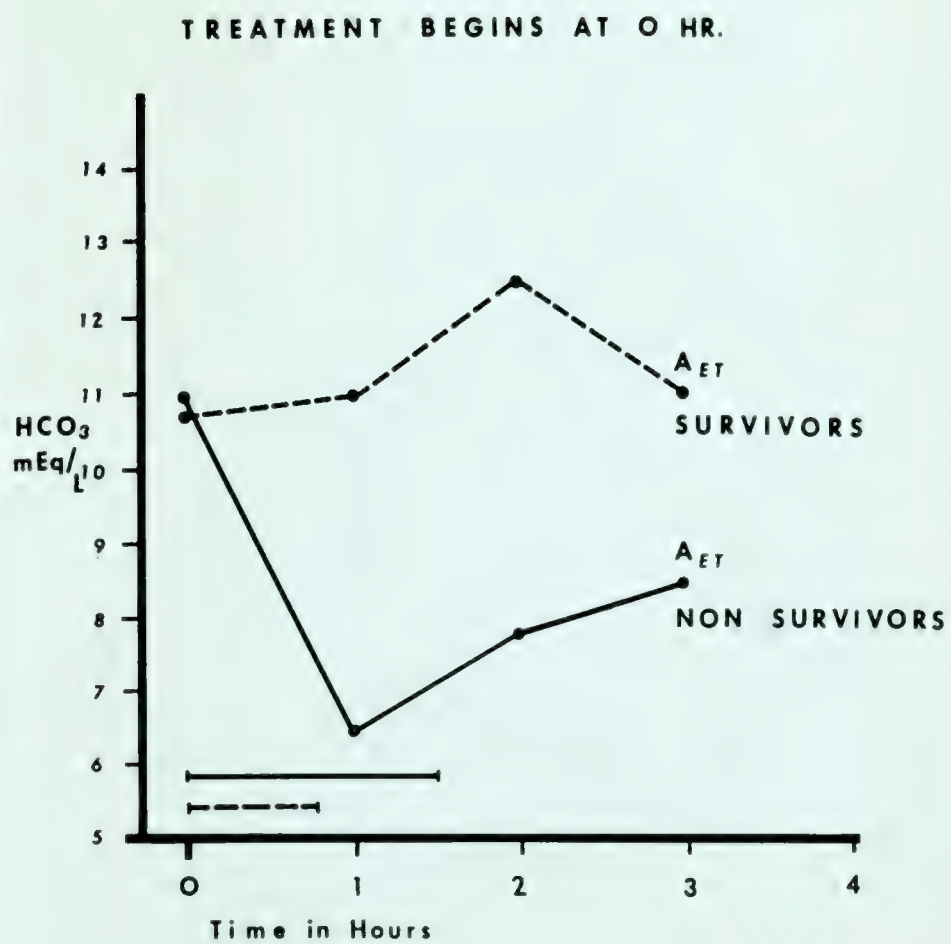
The results obtained by the combination of phentolamine and dibenzylene, in the absence of other measures, are not encouraging. It was hoped that the use of the rapidly acting adrenergic blocking agent would abolish the vasoconstrictor effect of endotoxin without delay and prevent pooling in the microcirculation. In the meantime, dibenzylene blockade would develop and during its long-lasting effect afford continued protection for the microcirculation. In 9 dogs out of 15 this regime in fact worked very well. In the remaining 6 dogs, however, marked deterioration was evident from the time treatment was instituted. It is possible that in these dogs deterioration was either directly caused or accelerated by the treatment given.

Flow-meter studies.

Use of the bubble flow meter, as described in Chapter II, required heparinization of the dog in order to eliminate clotting problems. The dose of heparin used (2 mg./Kg.) produced a marked bleeding tendency in some of the dogs. Bleeding was seen even from non-traumatized regions, the nostrils and the gingivae in particular. Dogs that bled excessively had to be disqualified as experimental subjects.

Figure 33.

HCO_3 in Treated Dogs



The need for heparinization precluded the study of flow after endotoxin, since heparin pretreatment has been reported to protect the dog significantly from the effects of endotoxin.

An interesting observation forthcoming from the flow studies was the profound hypotension produced by phentolamine and phenoxybenzamine, both individually and combined, in the presence of heparin. This observation is in marked contrast with the findings of the standard treatment control experiments in which no significant change in the blood pressure was observed. Nor could the hypotension be accounted for by haemorrhage, since it was also present in dogs that had not bled excessively externally, nor had evidence of internal haemorrhage at autopsy.

In view of the severe hypotension produced by phentolamine and phenoxybenzamine in the presence of heparin, no conclusions can be drawn regarding the influence of these drugs on flow. It is worthy of note, however, that when saline was infused after the administration of adrenergic blocking agents, mean blood pressure, pulse pressure, femoral artery flow and hind limb venous flow all showed marked, but temporary improvement.

These studies are illustrated by the figures below.
(Figs. 34, 35, 36, 37, 38).

Figure 34.

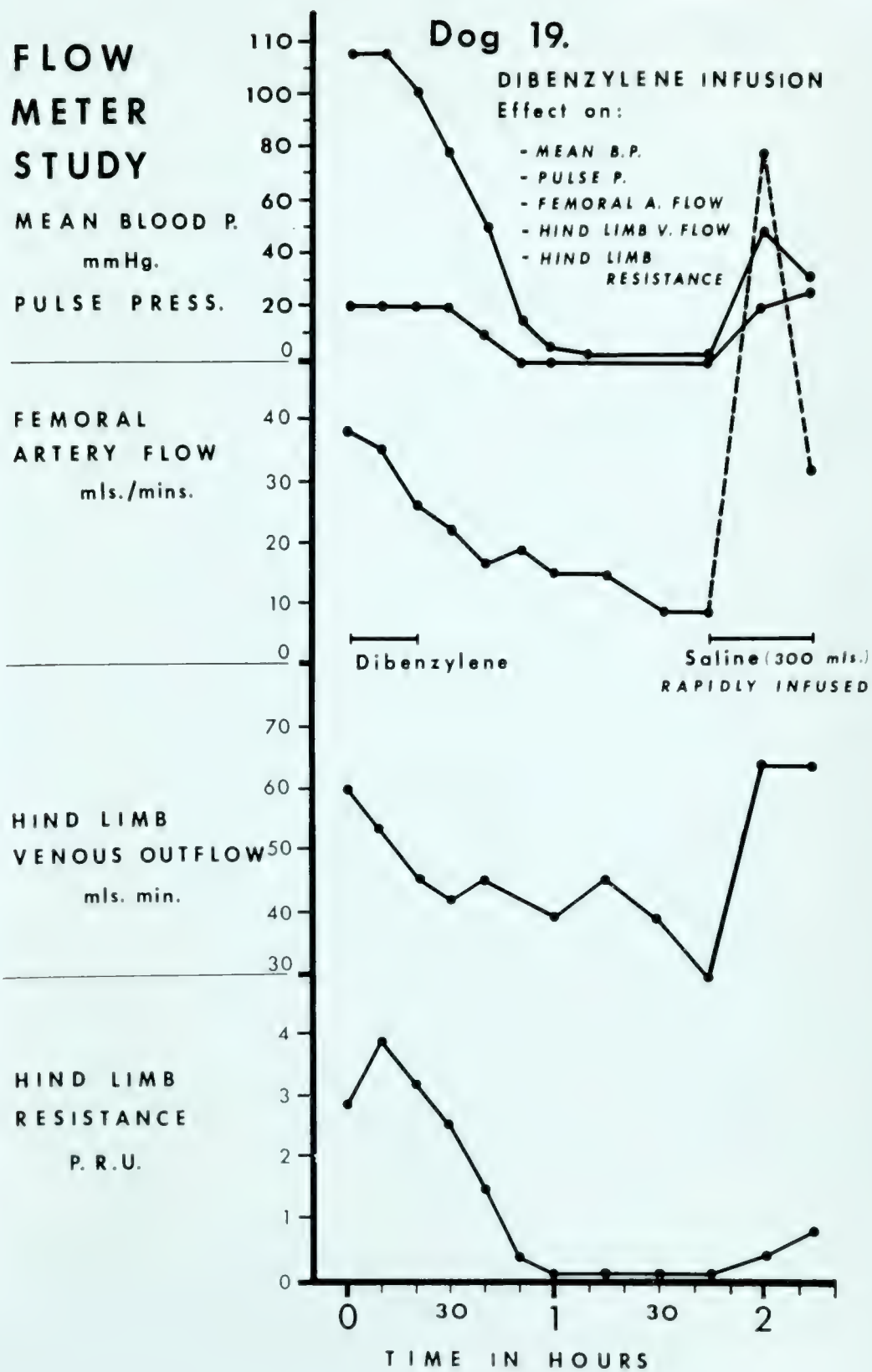


Figure 35.

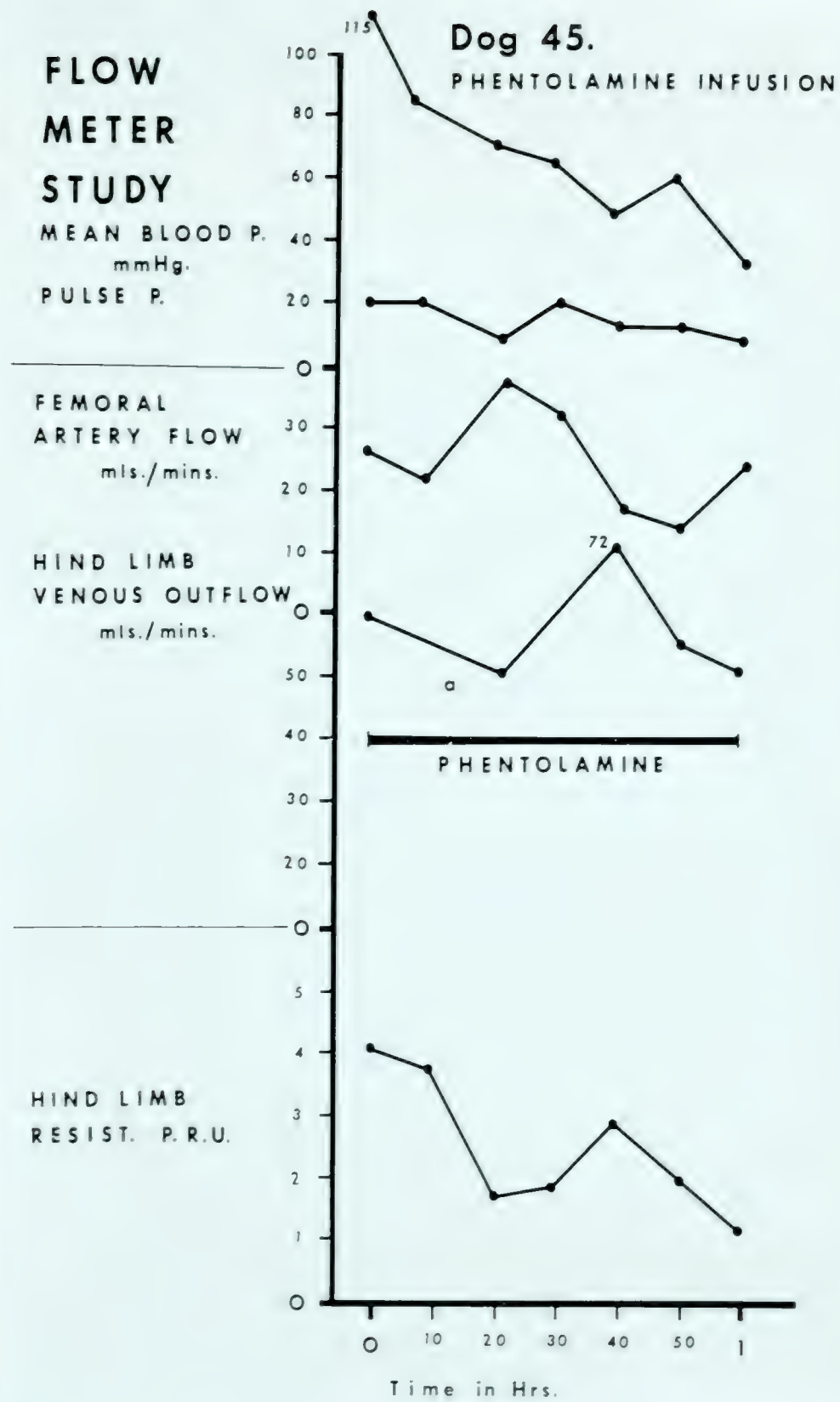


Figure 36.

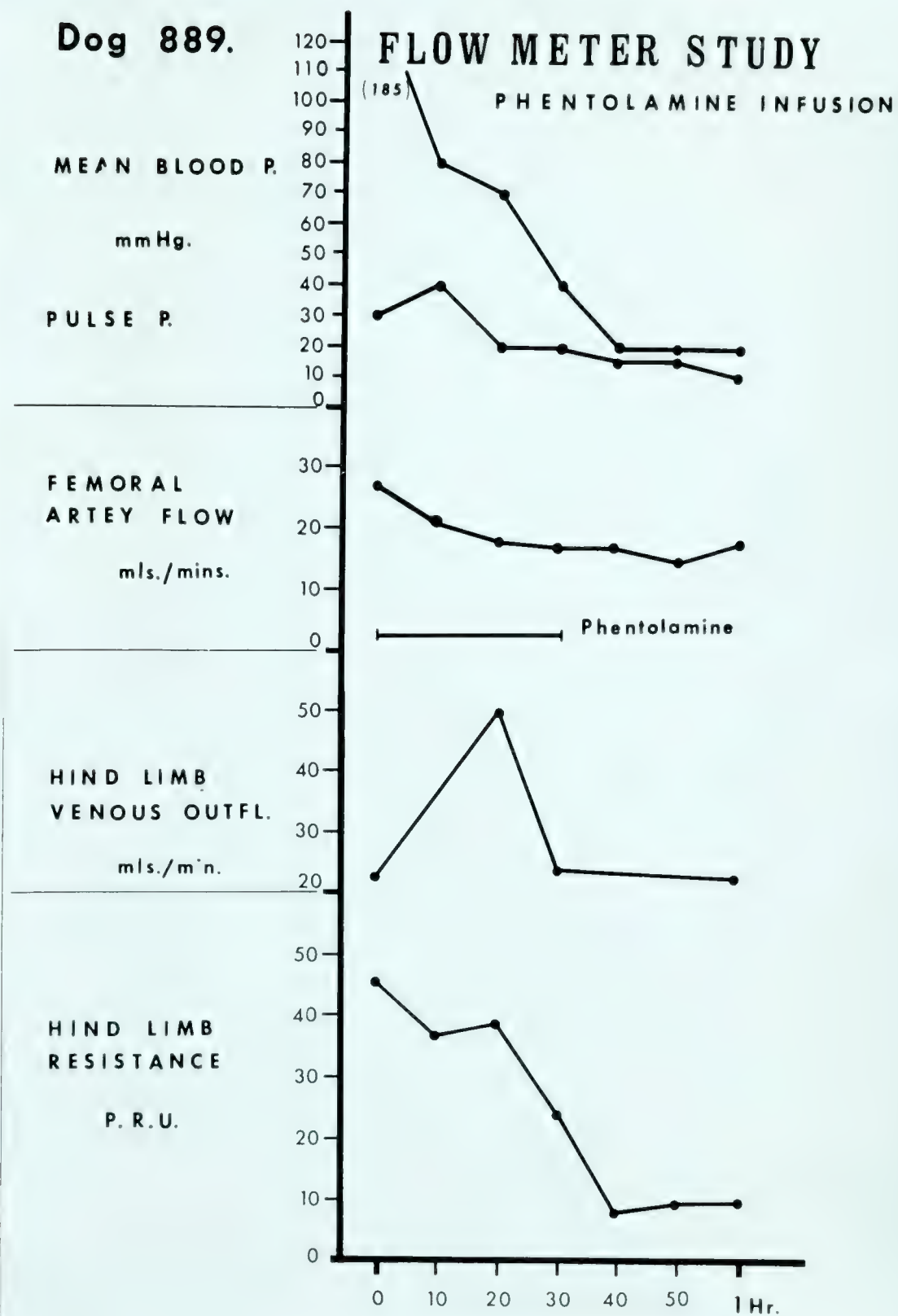


Figure 37.

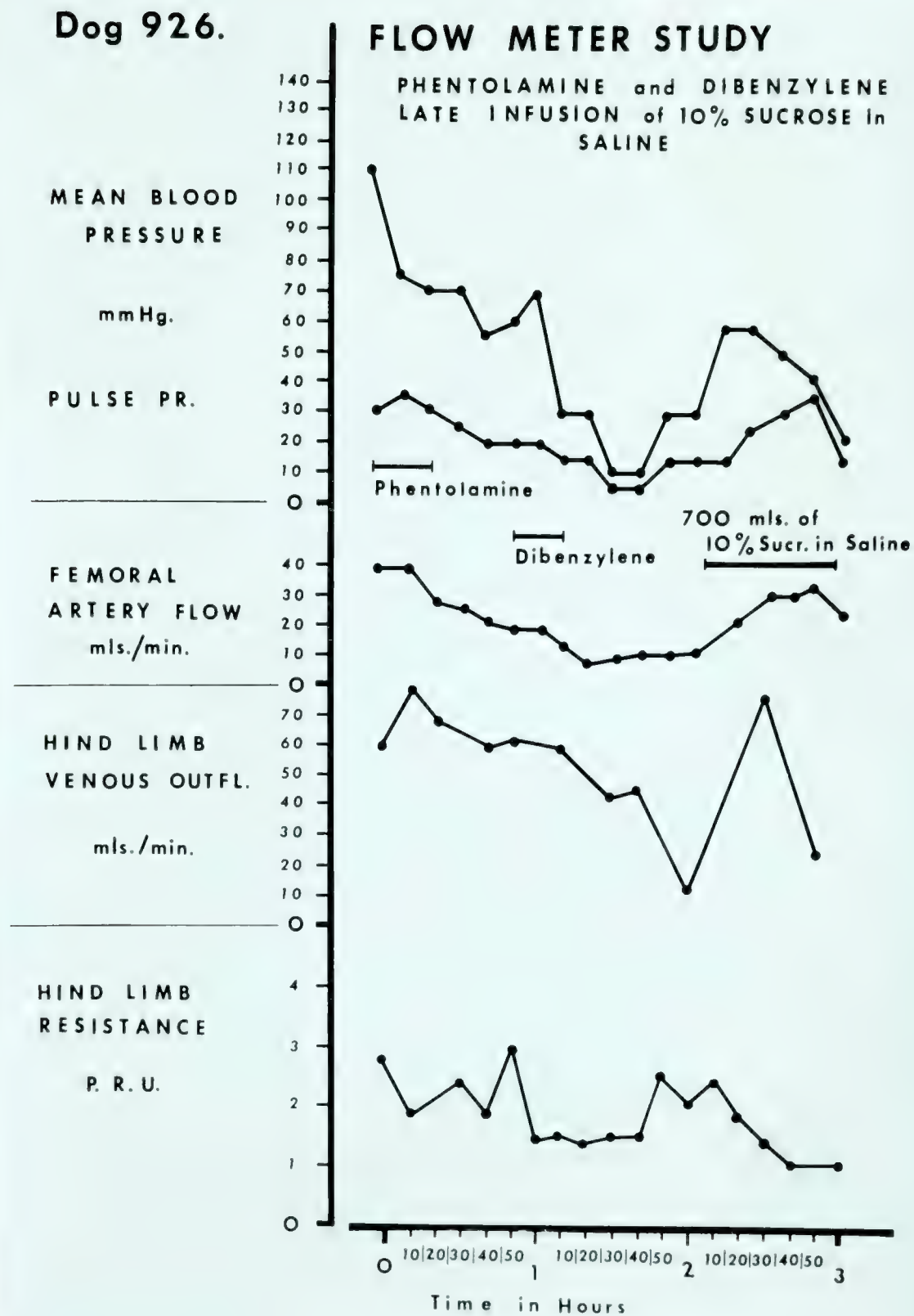


Figure 38.

**FLOW
METER
STUDY
Dog 48**

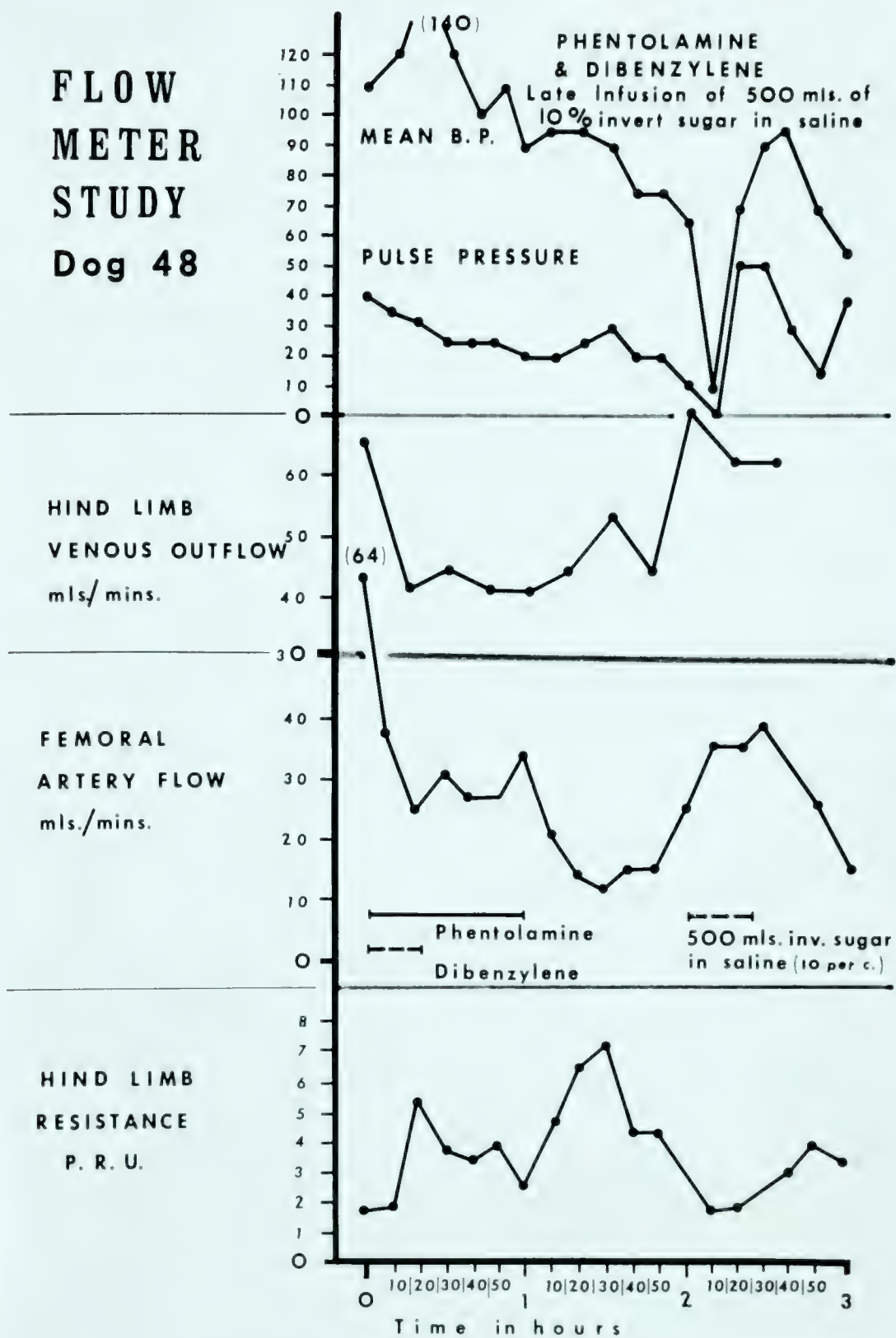


Figure 39.

U

	E			E(S)			T			ET		
	Date	No.	Survival	Date	No.	Survival	Date	No.	Survival	Date	No.	Survival
1	14 Aug	351	5.40 hrs	6 Mar	815	14 hrs	24 Feb	766	*	18 Oct	452	*
2	4 Sep	349	5.40 hrs	13 Mar	841	21 hrs	25 Feb	757	*	6 Nov	511	17.30 hrs
3	5 Nov	500	*	16 Mar	854	1.25 hrs	26 Feb	754	*	7 Nov	520	*
4	4 Feb	730	*	16 Mar	846	23 hrs	27 Feb	765	*	13 Nov	523	*
5	7 Feb	742	30 hrs							11 Mar	836	10 hrs
6	14 Feb	726	120 hrs							11 Mar	840	40 hrs
7	18 Feb	763	15 hrs							12 Mar	851	*
8	19 Feb	743	*							13 Mar	842	15 hrs

* indicates survival

RECORD OF EXPERIMENTS AND SURVIVAL

Figure 40.

A

	E			E(S)			T			ET		
	Date	No.	Survival	Date	No.	Survival	Date	No.	Survival	Date	No.	Survival
1	28 Nov	563	19 hrs	3 Mar	802	*	20 Nov	527	*	14 Nov	524	4.30 hrs
2	20 Jan	688	*	4 Mar	804	39 hrs	21 Nov	566	*	15 Nov	544	15 hrs
3	21 Jan	696	14 hrs	5 Mar	821	19 hrs	20 Feb	785	*	18 Nov	542	8 hrs
4	22 Jan	558	6.50 hrs	5 Mar	822	*	21 Feb	783	*	19 Nov	538	*
5	24 Jan	697	120 hrs				1 Apr	868	ATd	2 Mar	803	*
6	27 Jan	678	20 hrs				2 Apr	895	ATp	9 Mar	824	*
7	28 Jan	721	*				3 Apr	890	ATp	10 Mar	830	19 hrs
8	3 Feb	728	19 hrs							10 Mar	831	*
9	23 Mar	864	37 hrs				22 Apr	889	flow	8 Apr	896	*
10	24 Mar	859	*				23 Apr	926	flow	9 Apr	913	*
11	25 Mar	848	*				24 Apr	21	flow	13 Apr	909	12 hrs
12	6 Apr	875	*				27 Apr	33	flow	14 Apr	914	*
13	7 Apr	880	48 hrs				28 Apr	19	flow	1 May	47	48 hrs
14	16 Apr	929	120 hrs				29 Apr	48	flow	4 May	49	*
15	20 Apr	911	72 hrs				6 May	45	flow	5 May	62	*

* indicates survival

RECORD OF EXPERIMENTS AND SURVIVAL

S U M M A R Y A N D C O N C L U S I O N S

During the last fifty years, we have seen spectacular advances in the field of medicine. Yet in the same half-century we have not succeeded in reducing the high mortality from endotoxin shock. The increasing frequency with which this condition is being recognized in both surgical and medical practice, and the high mortality rate despite all our efforts to save these patients, place endotoxin shock high on the list of clinical problems to which a therapeutic answer must urgently be found.

The complex pathophysiological changes initiated by bacterial endotoxins have given rise to widely divergent interpretations of the pathogenesis of endotoxin shock, and many different methods of treatment, none of them wholly effective, have been suggested.

One of the basic difficulties confronting the clinician in this context is the fact that the human patient is not the most suitable subject for the study of the pathophysiology of endotoxin shock. A standardized study is possible only on the experimental animal. In animal studies, however, while we must accept the species difference, the experiment must be designed in a manner which will parallel the clinical situation.

The use of anaesthesia in experimental endotoxin shock is a deviation from the clinical parallel, since the human

patient is usually unanaesthetized. It has also been argued that anaesthetic agents alter the reactivity of blood vessels and thus may prejudice vascular responses to endotoxin. For this reason, experiments were conducted on both anaesthetized and unanaesthetized dogs. There was no significant difference in the results obtained in the two groups. Moreover, experiments on unanaesthetized dogs were found to present difficulties which limited the value of the observations made. Experiments conducted under anaesthesia were easier to perform and yielded more information. It is concluded that there is no advantage in the study of endotoxin shock in the unanaesthetized animal.

In all reports of endotoxin shock experiments in the literature so far, endotoxin was administered to animals by rapid injection of a single, massive dose. This method of endotoxin administration does not represent the development of endotoxaemia in man, since invasion of the bloodstream by endotoxin probably occurs over the course of a few hours. To achieve closer similarity to the clinical picture, in this study endotoxin was infused into dogs over a two hour period and the results were compared with those of a rapid injection. The slow infusion produced a steadily progressive deterioration in the parameters studied. In contrast with reports published so far it proves that the blood pressure of the dog in endotoxaemia behaves in the same manner as the blood pressure of man. The rapid injection of endotoxin, on the other hand, produced wide fluctuations

in the parameters which were confusing and more likely due to the method of administration than to the endotoxin itself. The conclusion is drawn, therefore, that in future studies in the dog more reliable results will be obtained by the slow infusion of endotoxin in a minimum fluid volume over a period of at least two hours.

There is a logical basis for the use of adrenergic blocking agents in endotoxin shock. The slow onset of action of dibenzylene, however, is a handicap in an emergency situation such as shock. For this reason, phentolamine was given in addition to produce rapid adrenergic blockade from the start of treatment, while waiting for dibenzylene to develop its sustained blockade. The results indicate that adrenergic blockade, in the absence of other treatment measures, gives no promise of success. While it produced improvement in some dogs, in others it may have accelerated the fatal outcome. The precept of "primum noli nocere" is not supported by this form of treatment. It would seem worthwhile, however, to combine adrenergic blockade, as used in this study, with volume replacement to compensate for the increase in vascular space mediated by sympathetic blocking action.

Traditions die slowly. The treatment of shock in clinical practice today is still much influenced by tradition. With the increasing understanding of the pathophysiological changes initiated by endotoxins, however, newer concepts of

treatment are evolving, which shift the clinician's interest from preoccupation with the blood pressure alone to consideration of the circulation as a whole. On this sound basis an effective answer to endotoxin shock will surely be found.

REFERENCES

1. Alexander, R. S., The participation of the venomotor system in pressor reflexes., *Circ. Res.* 2: 405, 1954.
2. Alican, F., Hardy, J. D., Sympatho-adrenal system in endotoxin shock., *Surg. Forum*, 13: 8, 1962.
3. Altemeier, W. A., Cole, W., Nature and treatment of septic shock., *A.M.A. Arch. Surg.*, 77: 498, 1958.
4. Altemeier, W. A., Cole, W., Septic shock., *Ann. Surg.*, 143: 600, 1956.
5. Annotations, *Lancet*, 1: 983, 1963.
6. Cannon, W. B., The emergency function of the adrenal medulla in pain and the major emotions., *Am. J. Physiol.*, 33: 356, 1914.
7. Cannon, W. B., *Traumatic Shock.*, D. Appleton Co., New York, 1923.
8. Cavanagh, D., DeCenzo, J. A., Endotoxin Shock. A promising new method of treatment based on animal studies., *Am. J. Obst. and Gynec.*, 85: 892, 1963.
9. Clein, L. J., Couves, C. M., An evaluation of treatment methods in experimental endotoxin shock., *Surg. Forum*, 13: 18, 1962.
10. Clein, L. J., Couves, C. M., Septic Shock, *C.M.A.J.*, 85: 1022, 1961.
11. Daniel, P. M., Prichard, M. M. L., Variations in the circulation of portal venous blood within the liver., *J. Physiol.*, 114: 521, 1951.
12. Davis, R. B., Meeker, W. R., Bailey, W. L., Serotonin release after injection of *E. coli* endotoxin in the rabbit., *Fed. Proc.*, 20: pt.1, 261, 1961.
13. Doberneck, R. C., Johnson, D. G., Hardaway, R. M., Blood volume adjustments to shock in dogs., *Arch. Surg.*, 86: 267, 1963.

14. Douglas, G. W., Beller, F. K., Debrovner, C. H., The demonstration of endotoxin in the circulating blood of patients with septic abortion., *Am. J. Obst. and Gynec.*, 87: 780, 1963.
15. Dripps, R. D., Anaesthesia and shock., *Fed. Proc.* 20: Suppl. 9, 224, 1961.
16. *Drugs of Choice.*, 1962-1963, C. V. Mosby Co., St. Louis, 1962.
17. Erlanger, J., Gesell, R., Gasser, H. S., Studies in secondary traumatic shock, I. The circulation in shock after abdominal injuries., *Am. J. Physiol.*, 49: 90, 1919.
18. Fine, J., *The Bacterial Factor in Traumatic Shock.*, Charles C. Thomas, Springfield, 1954.
19. Fine, J., Host resistance to bacterial infection in traumatic shock., *Brit. J. Anaesth.*, 30: 485, 1958.
20. Folkow, B., Nervous control of blood vessels., *Physiol. Rev.*, 35: 629, 1955.
21. Frank, H. A., Jacob, S., Friedman, E. W., Rutenburg, A. M., Glotzer, P., Fine, J., Irreversibility of haemorrhagic shock and VDM Hypothesis., *Am. J. Physiol.*, 168: 150, 1952.
22. Freeman, N. E., Decrease in blood volume after prolonged hyperactivity of the sympathetic nervous system., *Am. J. Physiol.*, 103: 185, 1933.
23. Freeman, N. E., Shaffer, S. A., Schechter, A. E., Holling, H. E., The effect of total sympathectomy on the occurrence of shock from haemorrhage., *J. Clin. Invest.*, 17: 359, 1938.
24. Freeman, N. E., Shaw, J. L., Snyder, J. C., The peripheral blood flow in surgical shock., *J. Clin. Invest.* 15: 651, 1936.
25. Gilbert, R. P., Mechanisms of the haemodynamic effects of endotoxin., *Physiol. Rev.*, 40: 245, 1960.
26. Gillenwater, J. Y., Dooley, E. S., Frohlich, E. D., Effects of endotoxin on renal function and haemodynamics., *Am. J. Physiol.*, 205: 293, 1963.
27. Goodman, L. S., Gilman, A., *The Pharmacological Basis of Therapeutics.*, MacMillan, New York, 1955.

28. Gourzis, J. T., Hollenberg, M. W., Nickerson, M.,
Involvement of adrenergic factors in the effects of
bacterial endotoxin., J. Exp. Med., 114: 593, 1961.
29. Green, H. D., Denison, A. B., Jr., Williams, W. O., Jr.,
Garvey, A. H., Tabor, C. G., Comparison of the potency
of dibenzylene, ilidar, phentolamine (regitine) and
tolazoline (priscoline) in blocking the vasoconstrictor
responses in canine muscle to lumbar sympathetic stimulation
and to intra-arterial injections of L-epinephrine and of
L-norepinephrine., J. Pharmacol. and Exp. Therapy.,
112: 462, 1954.
30. Green, H. D., Lewis, R. N., Nickerson, N. D., Quantitation
of changes of vasomotor tone. Change of vasomotor tone
as the cause of Traube-Hering waves., Proc. Soc. Exp.
Biol. Med., 53: 228, 1943.
31. Hakstian, R. W., Hampson, L. G., Gurd, F., Pharmacological
agents in experimental haemorrhagic shock., A.M.A. Arch.
Surg., 83: 335, 1961.
32. Hall, W. H., Gold, D., Shock associated with bacteraemia.,
A.M.A. Arch. Int. Med., 96: 403, 1955.
33. Hardaway, R. M., A new theory of shock., Military
medicine., 128: 198, 1963.
34. Hardaway, R. M., Barila, T. G., Burns, J. W., Mock, H. P.,
Studies on pH changes in endotoxin and haemorrhagic shock.,
J. Surg. Res., 1: 278, 1961.
35. Hardaway, R. M., Johnson, D., Clotting mechanism in
endotoxin shock., Arch. Int. Med., 112: 775, 1963.
36. Hardy, E. G., Morris, G. C., Yow, E. M., Haynes, B. W.,
DeBakey, M. E., Studies on the role of bacteria in
irreversible haemorrhagic shock in dogs., Ann. Surg.,
139: 282, 1954.
37. Herring, W. B., Herion, J. C., Walker, R. I., Palmer, J. G.,
Distribution and clearance of circulating endotoxin.,
J. Clin. Invest., 42: 79, 1963.
38. Heymans, C., Neil, E., Reflexogenic Areas of the Cardio-
vascular System., J. & A. Churchill, London, 1958.
39. Hinshaw, L. B., Kuida, H., Gilbert, R., Visscher, M. B.,
Influence of perfusate characteristics on pulmonary vascular
responses to endotoxin., Am. J. Physiol., 191: 293, 1957.

40. Hinshaw, L. B., Nelson, D. L., Venous response of intestine to endotoxin., *Am. J. Physiol.*, 203: 870, 1962.
41. Huckabee, W. E., Lactic Acidosis., *Am. J. Cardiol.*, 12: 663, 1963.
42. Iampietro, P. F., Hinshaw, L. B., Brake, C. M., Effects of an adrenergic blocking agent on vascular alterations associated with endotoxin shock., *Am. J. Physiol.*, 204: 611, 1963.
43. Jacob, S., Weizel, H., Korman, G. H., Schweinburg, F., Frank, H., Fine, J., Bacterial action in development of irreversibility to transfusion in haemorrhagic shock in the dog., *Am. J. Physiol.*, 179: 523, 1954.
44. Kirpekar, S. M., Cervoni, P., Effect of cocaine, Phenoxybenzamine and phentolamine on the catecholamine output from spleen and adrenal medulla., *J. Pharmacol. and Exp. Ther.*, 142: 59, 1963.
45. Kobold, E. E., Lucas, R., Thal, A. P., Chemical mediators in clinical septic shock., *Surg. Forum.*, 14: 16, 1963.
46. Lansing, A. M., Septic Shock., *Canad. Med. Ass. J.*, 89: 583, 1963.
47. Levy, E. R., North, W. C., Wells, J. A., Modification of traumatic shock by adrenergic blocking agents., *J. Pharmacol. and Exp. Ther.*, 112: 151, 1954.
48. Lillehei, R. C., The intestinal factor in irreversible haemorrhagic shock., *Surgery*, 42: 1043, 1957.
49. Lillehei, R. C., Discussion, Shock: Pathogenesis and Therapy., (Ciba Symposium) 1962.
50. Lillehei, R. C., Prevention of irreversible haemorrhagic shock in normal and Eck-fistula dogs by controlled cross-perfusion of the superior mesenteric artery., *Am. J. Physiol.*, 187: 614, 1956.
51. Lillehei, R. C., Longerbeam, J. K., Bloch, J. H., Physiology and therapy of bacteremic shock: Experimental and clinical observations., *Am. J. Cardiol.*, 12: 599, 1963.
52. Lillehei, R. C., MacLean, L. D., The intestinal factor in irreversible endotoxin shock., *Ann. Surg.*, 148: 513, 1958.

53. Lillehei, R. C., MacLean, L. D., Physiological approach to a successful treatment of endotoxin shock in the experimental animal., *A.M.A. Arch. Surg.*, 78: 464, 1959.
54. Longerbeam, J. K., Bloch, J. H., Manax, W. G., Lillehei, R. C., The treatment of irreversible shock., (Exhibit at 49th Clinical Congress of Am. Coll. Surg., San Francisco, Calif., 1963).
55. Longerbeam, J. K., Lillehei, R. C., Scott, W. R., The nature of irreversible shock., *A haemodynamic study.*, *Surg. Forum.*, 13: 1, 1962.
56. Lundholm, E., Effect of adrenaline on lactic acid content of rabbit blood., *Acta Physiol. Scand.*, 19: 1949, suppl. 67.
57. MacLean, L. D., Pathogenesis and treatment of bacteraemic shock., *Int. Abstr. Surg.*, 115: 307, 1962.
58. MacLean, L. D., Panel discussion, 49th Clinical Congress of the Am. Coll. of Surg., San Francisco, Calif., 1963.
59. MacLean, L. D., Shock due to sepsis - a summary of current concepts of pathogenesis and treatment., *J. Trauma.*, 2: 412, 1962.
60. Malcolm, J. D., The condition of blood vessels during shock., *Lancet*, 2: 573, 1905.
61. Mapother, E. D., Shock: its nature, duration and mode of treatment., *Brit. Med. J.*, 2: 1023, 1879.
62. McKay, D. G., The generalized Shwartzman reaction in the pregnant woman., *Obst. and Gynec.*, 20: 446, 1962.
63. Mellander, S., Comparative studies on the adrenergic neurohumoral control of resistance and capacitance blood vessels in the cat., *Acta. Physiol. Scand.*, 50: suppl. 176, 1960.
64. Moore, F. D., Relevance of experimental shock studies to clinical shock problems., *Fed. Proc.*, Suppl. 9: 227, 1961.
65. Moyer, J. H., Panel discussion: International Symposium on angiotensin, sodium and hypertension., *Can. Med. Ass. J.*, 90: 334, 1964.
66. Moyer, J. H., Handley, C. A., Huggins, R. A., The effect of adrenergic blockade and norepinephrine on renal and cardiovascular haemodynamics following haemorrhage., *Circ. Res.*, 2: 441, 1954.

67. Nash, C. W., Milligan, J. V., An automatic recording bubble flow meter, I.R.E. Transactions on Medical Electronics., ME-6: 274, 1959.
68. Nickerson, M., Factors of vasoconstriction and vasodilatation in shock., J. Mich. State Med. Soc., 54: 45, 1955.
69. Nickerson, M., Panel discussion, Am. J. Cardiol., 12: 628, 1963.
70. Nickerson, M., Drug therapy of shock; Shock: Pathogenesis and Therapy., (Ciba Symposium), Springer-Verlag, Berlin, 1962.
71. Nickerson, M., Sutter, M. C., Angiotensin in shock., Canad. Med. Ass. J., 90: 325, 1964.
72. Nickerson, M., Fed. Proc., (Suppl. 9), 204, 1961.
73. Nickerson, M., Sympathetic blockade in the therapy of shock., Am. J. Cardiol., 12: 619, 1963.
74. Nickerson, M., Carter, S. A., Protection against acute trauma and traumatic shock by vasodilators., Can. J. Biochem. Physiol., 37: 1161, 1959.
75. Nickerson, M., Gourzis, J. T., Blockade of sympathetic vasoconstriction in the treatment of shock., J. Trauma. 2: 399, 1962.
76. Palmerio, C., Zetterstrom, B., Shammash, J., Euchbaum, E., Frank, E., Fine, J., Denervation of the abdominal viscera for the treatment of traumatic shock., N. Eng. J. Med. 269: 709, 1963.
77. Peretz, D. I., McGregor, M., Dossetor, J. B., Lactic-acidosis: a clinically significant aspect of shock., Canad. Med. Ass. J., 90: 673, 1964.
78. Price, H. L., General anaesthesia and circulatory homeostasis., Physiol. Rev., 40: 187, 1960.
79. Rayner, R. R., MacLean, L. D., Grim, F., Intestinal tissue blood flow in shock due to endotoxin., Circ. Res., 8: 1212, 1960.
80. Remington, J. W., Hamilton, W. F., Boyd, G., Hamilton, W. F., Jr., Caddell, H. M., Role of vasoconstriction in the response of the dog to haemorrhage., Am. J. Physiol., 161: 116, 1950.

81. Rosen, F. S., The endotoxins of Gram-negative bacteria and host resistance., N. Eng. J. Med., 264: 919, 980, 1961.
82. Rosenberg, J. C., Lillehei, R. C., Longerbeam, J. K., Zimmermann, B., Studies on haemorrhagic and endotoxin shock in relation to vasomotor changes and endogenous circulating epinephrine, norepinephrine and serotonin., Ann. Surg., 154: 611, 1961.
83. Rubenstein, H. S., Fine, J., Coons, A. H., Localization of endotoxin in the walls of the peripheral vascular system during lethal endotoxaemia., Proc. Soc. Exp. Biol. Med., 111: 458, 1962.
84. Ruedy, J., Dirks, J. H., Cameron, D. G., Bacteraemic Shock - A Medical Emergency., C.M.A.J., 89: 1059, 1963.
85. Schayer, R. W., Relationship of induced histidine decarboxylase activity and histamine synthesis to shock from stress and from endotoxin., Am. J. Physiol., 198: 1187, 1960.
86. Schayer, R. W., Relationship of stress-induced histidine decarboxylase to circulatory homeostasis and shock., Science, 131: 226, 1960.
87. Schweinburg, F., Fine, J., Resistance to bacteria in haemorrhagic shock. II. Effect of transient vascular collapse on sensitivity to endotoxin., Proc. Soc. Exp. Biol. Med., 88: 589, 1955.
88. Schweinburg, F. B., Shapiro, P. B., Frank, E. D., Fine, J., Host resistance in haemorrhagic shock. IX. Demonstration of circulating lethal toxin in haemorrhagic shock., Proc. Soc. Exp. Biol. Med., 95: 646, 1957.
89. Selkurt, E. E., Rothe, C. F., Critical analysis of experimental haemorrhagic shock models., Fed. Proc. 20: Suppl. 9, 30, 1961.
90. Sheriton, J. E., White, A. G., Septicaemia due to Gram-negative organisms complicating vaginal repair., Brit. Med. J. 2: 1575, 1963.
91. Shorr, E., Zweifach, B. W., Furchgott, R. F., Baez, S., Hepatorenal factors in circulatory homeostasis., Circ., 3: 42, 1951.
92. Shubin, H., Weil, M. H., Bacterial shock. A serious complication in urological practice., J.A.M.A., 185: 850, 1963.

93. Siggaard-Andersen, O., The pH-logpCO₂ blood acid-base nomogram revised., Scand. J. Clin. Lab. Invest., 14: 598, 1962.
94. Siggaard-Andersen, O., Engel, K., A new acid-base nomogram., Scand. J. Clin. Lab. Invest., 12: 177, 1960.
95. Simeone, F. A., Shock, trauma and the Surgeon., Ann. Surg., 158: 759, 1963.
96. Sjostrand, T., Volume and distribution of blood and their significance in regulating the circulation., Phys. Rev., 33: 202, 1953.
97. Spink, W. W., Endotoxin Shock., Ann. Int. Med., 57: 538, 1962.
98. Thomas, L., Role of epinephrine in reactions produced by endotoxins of Gm-ve bacteria, I., J. Exp. Med., 104: 865, 1956.
99. Thomas, L., Stetson, C. A., Studies on the mechanism of the Shwarzman Phenomenon, J. Exp. Med., 89: 461, 1949.
100. Thomas, L., Zweifach, B. W., Benacerraf, B., Mechanisms in the production of tissue damage and shock by endotoxins, Trans. Ass. Am. Physicians, 70: 54, 1957.
101. Thomas, W. D., Essex, H. E., Observations on the hepatic venous circulation with special reference to the sphincteric mechanism., Am. J. Physiol., 158: 303, 1949.
102. Udhoji, V. N., Weil, M. H., Sambhi, M. P., Roboff, L., Haemodynamic studies on clinical shock associated with infection., Am. J. Med., 34: 461, 1963.
103. Vick, J. A., Lafave, J. W., MacLean, L. D., The effect of treatment of endotoxin shock on renal haemodynamics and survival., Surgery, 54: 78, 1963.
104. Walters, G., McGowan, G. K., Pulmonary embolism or bacterial shock., Lancet, 2: 17, 1963.
105. Weale, F. E., A simplified theory of shock. The fundamental role of the myocardium., Lancet, 1: 973, 1963.
106. Webster, M. E., Clark, W. R., Significance of the callicrein-callidinogen-callidin system in shock., Am. J. Physiol., 197: 406, 1959.

107. Webster, M. E., Sagin, J. F., Landy, M., Johnson, A., Studies on the "O" antigen of *Salmonella typhosa*., J. Immunol., 74: 455, 1955.
108. Weil, M. H., Miller, B. S., Experimental studies on therapy of circulatory failure produced by endotoxin., J. Lab. and Clin. Med., 57: 683, 1961.
109. Weil, M. H., Miller, B. S., Studies on the effects of a vasopressor agent, sympatholytic drugs, and corticosteroid in shock caused by bacterial toxin., Circ., 22: 830, 1960.
110. Weil, M. H., Miller, B. S., Observations on the development of acidosis and the effect of corticosteroid in shock due to endotoxin., Clin. Res., 7: 271, 1959.
111. Weil, M. H., Udhoji, V. N., Allen, K. S., The head down position in the treatment of shock., Surg. Gynaec. and Obst., 116: 669, 1963.
112. White, C., Use of ranks in a test of significance for comparing two treatments., Biometrics 8: 33, 1952.
113. Wigger, H. C., Ingraham, R. C., Roemhild, F., Goldberg, H., Vasoconstriction and the development of irreversible haemorrhagic shock., Am. J. Physiol., 153: 511, 1948.
114. Wilcoxon, F., Individual comparisons by ranking methods., Biometrics, 1: 80, 1945.
115. Wilson, J. N., Management of acute circulatory failure., Surg. Clin. N. Am., 43(2): 469, 1963.
116. Zweifach, B. W., Microcirculatory derangements as a basis for the lethal manifestation of experimental shock., Brit. J. Anaes., 30: 466, 1958.
117. Zweifach, B. W., Aspects of comparative physiology of laboratory animals relative to the problem of experimental shock., Fed. Proc., 20: Suppl. 9, 18, 1961.
118. Zweifach, B. W., The Functional Behaviour of the Microcirculation., Charles Thomas, Springfield, 1961.
119. Zweifach, B. W., Benacerraf, B., Thomas, L., The relationship between the vascular manifestations of shock produced by endotoxin, trauma and haemorrhage., J. Exp. Med., 106: 403, 1957.

120. Zweifach, B. W., Gordon, H. A., Wagner, M., Reyniers, J. A., Irreversible haemorrhagic shock in germfree rats., J. Exp. Med., 107: 437, 1958.
121. Zweifach, B. W., Lee, R. E., Hyman, C., Chambers, R., Omental circulation in morphinized dogs subjected to graded haemorrhage., Am. Surg., 120: 232, 1944.
122. Zweifach, B. W., Nagler, A. L., Thomas, L., Role of epinephrine in reactions produced by Endotoxins of Gram-negative bacteria., J. Exp. Med., 104: 881, 1956.

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